ARMY MEDICAL LIBRARY
FOUNDED 1836

U.S. NATIONAL
LIBRARY
OF
MEDICINE

HISTORY OF THE OFFICE OF
SCIENTIFIC RESEARCH AND DEVELOPMENT

A summary of the activities of the entire organization in the development of improved weapons of warfare has been published as *Scientists Against Time* by James Phinney Baxter, 3rd. Details about the different parts of the organization are presented in a series of volumes with the common title, *Science in World War II*, which has been prepared under authority from:

Vannevar Bush, President, Carnegie Institution of Washington
Director, Office of Scientific Research and Development

James B. Conant, President, Harvard University
Chairman, National Defense Research Committee

Alfred N. Richards, Vice-President in Charge of Medical Affairs, University of Pennsylvania
Chairman, Committee on Medical Research

Karl T. Compton, President, Massachusetts Institute of Technology
Chief, Office of Field Service

Science in World War II

NEW WEAPONS FOR AIR WARFARE
DIVISIONS 4, 5, AND 7 OF NDRC; SECTION T, OSRD

COMBAT SCIENTISTS
OFFICE OF FIELD SERVICE; NALOC; DOLOC

ADVANCES IN MILITARY MEDICINE
COMMITTEE ON MEDICAL RESEARCH

ROCKETS, GUNS, AND TARGETS
DIVISIONS 1, 2, AND 3 OF NDRC

CHEMISTRY
DIVISIONS 8, 9, 10, 11, 19 AND TDAC OF NDRC

APPLIED PHYSICS: ELECTRONICS; OPTICS; METALLURGY
DIVISIONS 13, 15, 16, 17, 18 AND COMMITTEE ON
PROPAGATION OF NDRC

ORGANIZING SCIENTIFIC RESEARCH FOR WAR
ADMINISTRATIVE FRAMEWORK OF OSRD

ADVANCES IN MILITARY MEDICINE

VOLUME II

Advances in Military Medicine

MADE BY AMERICAN INVESTIGATORS

WORKING UNDER THE SPONSORSHIP OF

THE COMMITTEE ON MEDICAL RESEARCH

EDITED BY

E. C. Andrus   C. S. Keefer

D. W. Bronk   J. S. Lockwood

G. A. Carden, Jr.   J. T. Wearn

M. C. Winternitz

ASSOCIATE EDITOR
Tuckerman Day

FOREWORD BY
Alfred N. Richards

VOLUME II

With Illustrations

PUBLISHER'S NOTE

Under the terms of the contract for the publication of *Advances in Military Medicine,* Volume II, and of the other volumes in the long history of the activities of the Office of Scientific Research and Development, entitled *Science in World War II,* the publisher has agreed to waive its right under the copyright of each separate volume after ten years from the date of publication of such volume. Thereafter the volume in question will be in the public domain and dedicated to the public.

This history of OSRD is intended to be a survey of the way in which certain weapons were developed, improved, or brought into use and medical knowledge was extended and applied to military situations. It is not intended to be a complete documentary record of the making of the inventions that happen to be mentioned in it. Therefore the dates used, which serve to outline the general chronology, do not necessarily establish dates of conception of inventions, of their reduction to practice, or of occasions of first use. Moreover, in the interest of simplicity, credit for the development of a device is frequently given to a group identified by the name of its leader, rather than to the individual who was the actual inventor.

The authors and editors of this volume receive no royalty from its sale.

CONTENTS

ILLUSTRATIONS

chiolar and alveolar distention, and normal-appearing epithelial cells. × 225.

D: Like A, but no HMT prophylaxis. Note marked perivascular edema, moderate dilatation of terminal bronchiole with low cuboidal epithelium and condensation of nuclei, and emphysema. × 225. (This microscopic appearance of bronchial epithelium is rare at this concentration of diphosgene but is typical at the high ones.)

E: Same conditions as A, but animal was sacrificed at 48 hours. Note lack of dilatation, lack of perivascular edema, columnar epithelium standing in folds, many young epithelial cells, and no marked peribronchiolar infiltration by inflammatory cells. × 225.

F: Same conditions as E, but no HMT prophylaxis. Note dilatation of terminal bronchiole, necrosis and sloughing of bronchiolar epithelium, little or no epithelial regeneration, accumulation of peribronchiolar inflammatory cells, and edema of perivascular connective tissue. × 225.

ADVANCES IN MILITARY MEDICINE

VOLUME II

PROBLEMS OF NUTRITION

JOHN B. YOUMANS AND GEORGE M. GUEST

NEW PROBLEMS of nutrition were presented by the expanding military services and various civilian agencies in the periods immediately preceding and during the war. These problems arose from recent developments in nutritional science, special conditions of operation and supply of our armed forces, shortages of nutrients, needs of civilian populations at home and abroad, and the necessity of realizing to the utmost whatever benefits might be obtained from proper knowledge and management of natural food supplies and supplements of accessory food factors that were available.

Preparations for the study of these problems were initiated in July 1940, when the Subcommittee on Medical Nutrition of the Committee on Medicine of the National Research Council was formed at the request of the Surgeons General of the Army and Navy. This committee was to provide information, recommendations, and advice on matters of nutrition, particularly its medical aspects, to the Co-ordination Committee on Nutrition of the Commission of National Defense and other agencies of the government, as well as to the Army and Navy. It functioned also as a subcommittee of the Food and Nutrition Board of the Division of Biology and Agriculture, National Research Council.

During 1940 and 1941 many subjects were presented to the committee for debate: the nutrient qualities of the Army rations then planned, minimal requirements for emergency rations, the possible need for supplementation of rations with vitamins under varying conditions, the possibility of supplying dehydrated foods to troops where this would simplify transportation, the salt and water requirements of men in hot climates, problems of fatigue in relation to diet and to the nutritional status of men under different working conditions, and criteria for the recognition of nutritional deficiency states in men of the military services and the members of large civilian groups for whom the military services might become responsible. When the Committee on Medical Research was formed in the fall of 1941, the Subcommittee on Medical Nutrition continued to serve in an advisory capacity, recommending desirable projects to the Committee and holding conferences in which investigators and military liaison representatives met to discuss work in progress and practical applications of new information as it was gained.

The OSRD(CMR) program of research in fields of human and animal nutrition developed along four main lines, concerned with food composition and preparation, diagnostic methodology, physical and mental fitness of men in relation to nutritional factors, and the chemistry of vitamins. In many of these investigations the information sought could be obtained only by controlled experiments on human subjects. Such studies were made possible by men who voluntarily served in experiments. The latter often involved hard work, much physical discomfort, tedious days of living in close quarters under prescribed conditions in extremely hot or cold, humid or dry environments, and acceptance at the same time of limited diets, which in many instances were continued at starvation or semistarvation levels for long periods of time.

Students, soldiers, and hospital patients served thus in some of the projects, but in most cases the subjects were conscientious objectors who volunteered their services and were assigned to the respective projects from Civilian Public Service Camps. In a project at the University of Minnesota, for example, 65 of these men were resident subjects in the laboratory for a period of one year.

FOOD COMPOSITION AND PREPARATION

The first need in the nutrition research program was for complete information on the nutrient qualities of foods commonly used or proposed for use by the military forces in different parts of the world. Abundance, caloric value, and taste appeal had to be considered, along with content of vitamins and minerals and other essentials of diets planned for use under widely varying circumstances.

Four groups of investigators studied, each by a somewhat different approach, the preservation or loss of nutritive value of foods through successive steps of various methods of preparation and cooking. Detailed analyses were made of the vitamin content of foods in the fresh state and before and after dehydration, canning, or other processing for their preservation, and of the same foods immediately after cooking by various methods and after holding them for varying periods, as they would be held in practice before serving. Such studies were made on a small laboratory scale at Cornell University, with attention to canned as well as dehydrated vegetables. In the laboratory of the Bureau of Human Nutrition and Home Economics of the United States Department of Agriculture, at Beltsville, Maryland, analyses of food served in a typical Army mess were made, with food samples obtained from the Army Auto Ordnance School at Fort Meade. Another study was conducted at the Pentagon Post Restaurant in Washington to determine the nutrient content of foods served in a large-scale operation, with the special objective of determining the stages in the preparation of foods at which the

loss of nutrients is greatest, and to devise means whereby those losses might be minimized by practical revisions in restaurant practice. The analyses included, for example, determinations of weight changes of vegetables and losses of ascorbic acid during large-scale cooking. The data thus gained were assembled in specially prepared tables and made available to nutritionists. At the University of California, a study was conducted of various procedures for dehydrating foods such as vegetables, fruits, and meats, with attention to factors influencing the preservation of various vitamins in the products and those affecting the palatability of the foods. The information gained in that study is especially valuable in improving methods for the large-scale preparation of dehydrated foods. These four projects operated under the supervision of the Subcommittee on Food Composition of the Food and Nutrition Board, Division of Biology and Agriculture, National Research Council.

To gather information on fats with a high melting point, which were used as shortening in rations for the military forces in tropical areas, an investigator at the University of Pittsburgh conducted extensive studies on the physical and chemical nature of these fats, with special regard to their digestibility and to characteristics responsible for the development of rancidity during prolonged storage at tropical temperatures. Much attention was paid to factors influencing the development of rancidity in solid food products, such as those made from the highly nutritious "full-fat" soybean flour. These studies were made in close consultation with the Military Planning Division and Subsistence Laboratory of the Office of the Quartermaster General and with commercial manufacturers of Army rations.

Studies to determine the so-called "biologic value" of various food proteins in human nutrition were conducted at the University of Rochester, in order to gain information that might guide the choice of proteins provided in rations for the armed forces and to ascertain the nutrient value of some of the cheaper sources of protein available to populations in foreign areas where problems of famine relief were anticipated.

The essential data were obtained by determinations of the nitrogen intake in the food and the nitrogen output in the urine and feces, with subjects first on a basal diet containing no protein and then with the tested protein added to the basal diet. The amount of protein given was sufficient approximately to establish nitrogen equilibrium under the conditions of the experiment. By such tests the biologic value of a given protein is expressed in percentage of the absorbed protein that is retained; thus, for a perfect protein a biologic value of 100 would indicate that all the nitrogen of the tested protein absorbed from the intestines was retained.

Nine different diet squads, with six to twelve volunteer subjects in each, were put through a series of feeding experiments lasting from seven to eleven weeks. Biologic values for whole-egg protein and soybean and other

proteins were determined at different levels of intake. Representative biologic values reported for a few of the proteins tested were: whole-egg protein (taken as the standard of reference as a most complete natural protein), 97; cottonseed protein, 92; yeast protein, 87; beefsteak protein, 84; soybean protein, 81; and peanut protein, 82.

Another objective of these experiments was to learn to what extent the biologic value of certain proteins could be accounted for by the ten essential amino acids, and to learn whether it would be possible to obtain the biologic value of beefsteak, for example, either by feeding a mixture of the amino acids or by supplementing a poor protein by selected amino acids. The investigators found that biologic values for several proteins and for mixtures of essential amino acids made up to imitate them did not agree. The discrepancy was accounted for by the *dl* forms of the seven amino acids (synthetic) that were available only in this form. These acids were isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane, and valine. A method was devised to correct for the unnatural isomers. By running an experiment in which the fed mixture contained a sufficient amount of the natural isomers of the essential amino acids to correct for the unnatural ones, correct biologic values were obtained. Such results suggest that the administration of synthetic amino acids by intravenous injection as a substitute for proteins that the body needs in emergencies would be extremely wasteful.

The same conclusion was reached in the use of single amino acids added as supplements to a diet containing an inadequate amount of whole-egg protein, in comparison with an extra amount of egg protein itself. The basic amino acids used as natural isomers were retained to the extent of 100 per cent (average) of the nitrogen fed, whereas the nitrogen of the *dl* synthetic forms was retained only to the extent of 60 per cent (average).

Included in the research on food composition was a study on rations for horses, conducted at the request of the Army Veterinary Corps. This study, done in the Bureau of Animal Husbandry of the Department of Agriculture, was designed to determine to what extent the rations of Army horses might be reduced in bulk and weight and still maintain minimal roughage requirements. The results indicated that the hay ration of horses could safely be reduced to one fifth the usual allowance, and that a complete ration in a compressed form (pellets or briquettes) with a roughage content of 33 per cent could be made that would maintain horses in good condition.

DIAGNOSTIC METHODOLOGY

The diagnosis of avitaminosis in ill patients who present obvious signs and symptoms of deficiency disease is easy, but the recognition of subtler manifestations of mild deficiencies in apparently healthy men is difficult. Several projects were directed primarily at the establishment of exact criteria

for the definition of borderline deficiencies and at the improvement of existing methods for evaluating them, especially methods suitable for field use in mass surveys for evaluating the nutritional status of large population groups. Work of this sort was also included in several of the projects mentioned in the next section, under studies of physical fitness in relation to nutritional factors.

Simplified methods for estimating various B vitamins in urine, suitable for use in nutrition surveys of large population groups, were developed at Duke University. These methods were employed in a rapid test devised for measuring relative bodily saturation or depletion of the B vitamins. This test involves merely the giving of a single oral dose of 5 mg. of thiamine, 5 mg. of riboflavin, and 50 mg. of nicotinamide and analyses of the two-hour urinary excretion of the three vitamins. To demonstrate the suitability of the method for surveys and to collect data on sample population groups, the test was applied to a large number of medical students, hospitalized patients, normal pregnant women, and so forth. It was found that ill patients as a group excreted much smaller amounts than did normal controls. This indicates either a greater demand for the B vitamins in disease or a higher rate of their destruction. With cessation of OSRD(CMR) support at the end of the war, the investigation is being continued under grants from the United States Public Health Service and private foundations. New methods are being elaborated for the estimation of niacin derivatives in blood and tissues, as well as interrelationships between proteins, amino acids, and vitamins. The methods are being applied to the study of vitamin saturation in various pathologic conditions.

At the Public Health Institute in New York, there was developed an ingenious system of microanalysis whereby a single blood sample of only 0.2 cc., obtained by skin puncture, served for determinations of hemoglobin, serum protein, phosphatase, and vitamins A and C. Also, field methods for the determination of urinary ascorbic acid, thiamine, and riboflavin were developed and adapted for use in testing men in tropical areas who were receiving quinacrine. An Army medical officer received instruction in the use of these methods for a survey mission in the Pacific area.

On the basis of his earlier studies of relationships observed between lowered serum alkaline phosphatase and scurvy in infants and children and in laboratory animals, an investigator at the Massachusetts Institute of Technology attempted to develop a serum-phosphatase test to detect mild or incipient scurvy. Although further valuable data were gathered on infantile and experimental scurvy, the test was not found to afford diagnostic aid in adult subjects with mild deficiency.

INFLUENCE OF NUTRITIONAL FACTORS

A number of projects were devoted to evaluating the influence of various nutritional factors on physical and mental efficiency, with special regard to deterioration under stress of partial deficiencies, starvation, fatigue, and abnormal environmental conditions. In many instances these investigations involved some duplication of effort because of overlapping interests in the physiological problems common to each. Such duplication of work proved advantageous when confirmatory findings on secondary problems were obtained independently by different investigators whose primary objectives were not closely related, and also when discrepancies in results from separate experiments could be expected to disclose fallacious interpretations of data.

In 1940 the German conquest of Norway and the Low Countries focused attention on the tactical role of parachutists and on questions of subsistence affecting the endurance of shock troops. There was obvious need for compact rations for individual issue, and it was suggested that the stamina of such forces might be helped by the use of vitamin "supercharging." Research on these questions was started early in 1941 at the Laboratory of Physiological Hygiene of the University of Minnesota, with assistance provided by the Army and private foundations and industries. OSRD(CMR) sponsorship was provided in the fall of 1941 in one of the first contracts made, and was continued throughout the war for work on many ramifying problems. In this project from the outset controlled human experiments were employed in practically all the tests of the efficacy of various dietary regimes and of the effects of deprivation on physical and mental fitness.

An early result of the collaboration between the Minnesota laboratory and the Office of the Quartermaster General was Ration K, first tested as a ration for parachutists, which became the major combat and emergency ration of the American military forces. Another early result was the demonstration that daily dosing with extra vitamins over periods of weeks or months conferred no significant advantage on normal American soldiers subsisting on regular Army rations. These findings provided reassurance concerning these Army rations, and by helping to counteract extravagant enthusiasm obviated the necessity of costly programs for supplementary administration of vitamin pills.

A principal question with regard to the planning of military rations was what criteria should be used to assure nutritional excellence of food, aside from abundance and taste appeal. The Recommended Daily Allowances of the National Research Council afforded a convenient measure, but one that might well be both impractical and unnecessarily generous. The Minnesota laboratory undertook to study the possible effects of moderate restrictions, for periods of weeks and months, of the intakes of the B vitamins. The

criteria for judgment were exhaustive tests and analyses of physical and mental performance, as well as studies in physiological and biochemical functions. Results reported in 1941 and 1942 were fully borne out by studies continuing through 1945; for periods at least as long as six months, the recommended allowances for thiamine, riboflavin, and niacin were something like twice as high as necessary for typical Army personnel. Studies with diets essentially devoid of B vitamins helped to clarify the picture of true vitamin deficiency and established the crucial role of thiamine in situations where only minute amounts of vitamins might be available.

Experiments with a group of 12 volunteer subjects were devised and carried on for over a year and half to study the effects of various prolonged and severe stresses on thiamine requirements and to answer the frequently posed questions whether the bodily needs for thiamine might be increased by physiological or metabolic stresses, and whether larger intakes of this vitamin would aid the body in emergencies. The stresses applied, with intervening rest periods of a month or two, included physical work at 120° F., subsistence on high-fat diets, lack of sleep, extremely hard work, induced malaria, and work without food for several days. It was found that even when the stresses were applied so as to produce marked physical deterioration, there was little if any demonstrable disadvantage to a daily intake of 1 mg. of thiamine compared with one of 2 mg.

In studies of nutritional factors affecting the physical state of men living in a special chamber under simulated tropical conditions, it was found that previously expressed fears of significant losses of vitamins in the sweat were groundless, even in a person sweating as much as 10 l. a day. Furthermore, high vitamin intakes did not improve the resistance of the body to heat. Vitamin losses in rations stored at high temperatures were found to be serious, particularly with regard to thiamine and ascorbic acid, and these findings led to the adoption of better facilities for storing foods in the field. The need in hot weather for abundant water was readily demonstrated, but the need for salt proved to be less than expected. Salt tablets were deemed unnecessary provided regular meals, fairly well salted, were eaten.

In 1944 the Minnesota laboratory was collecting information on the effects of hard work without food. It was logical to extend this work to conditions encountered with famine, since it appeared certain that the war would bring great problems of semistarvation and food relief, and that detailed knowledge on the results of semistarvation and on the requirements for subsequent nutritional rehabilitation would be sorely needed. The Office of Scientific Research and Development and the Office of the Surgeon General of the Army joined in sponsorship provided by a group of private foundations, industries, and churches to support a program that involved 36 young men as volunteer subjects and occupied more than a year in full operation, with follow-up studies continuing until the late summer of 1946. Under controlled

conditions these men were first standardized on a good American diet for months, then placed on a European type of famine diet for six months, and finally studied over many months of rehabilitation with feeding at different levels of intake. A quarter of the original body weight was lost, in spite of the development of considerable famine edema. Effects on work capacity, co-ordination, and intellective functions were measured. Significant relations were found between emotional states and the physiological changes. The exhaustive physiological, biochemical, and psychological findings provide a detailed and quantitative picture of sociological as well as direct medical consequence. Vitamins were found to be relatively unimportant in this starvation. It was shown that potatoes and whole-wheat bread can form the basis for a surprisingly good diet. The results of these starvation studies have been utilized by private relief organizations as well as by the agencies of a number of governments, and the proof that a long period of high-caloric feeding is needed for full rehabilitation has been applied in international food programs.

At the University of Tennessee, an attempt was made to determine what type of diet might be expected to condition the body to withstand the stress of abrupt starvation and might be provided if, in some circumstances, such a risk could be anticipated; for example, the risk faced by naval and air force personnel of being wrecked without provisions. A group of volunteers were subjected to three-day periods of starvation after ten-day preparatory periods during which they were given high-protein, high-carbohydrate, or high-fat diets, each approximately equicaloric and with ample vitamins. Chemical studies and measurements of response to standard exercise tests revealed no significant differences in ability to withstand fasting as affected by these diets.

At the University of Illinois, a research team carried out studies to determine the magnitude and significance of losses of water-soluble vitamins and minerals in the perspiration of adult human subjects under simulated tropical environmental conditions and with moderate work. Healthy young men served as subjects of the experiments, which involved exposures for periods of eight hours, five days a week, in an air-conditioned chamber, with temperature and humidity varied to simulate hot-dry desert and hot-moist jungle conditions. Data were collected on the rate of sweating with varying environmental conditions and with different levels of water intake. The sweat when analyzed was found to contain all the water-soluble vitamins, but the concentrations were so small as to represent insignificant losses from the standpoint of practical nutrition. These investigators found, in agreement with others, that the concentration of sodium chloride in the sweat tended to decrease with acclimatization.

In another University of Illinois project, experiments were undertaken in the Department of Medicine to determine the effect of dietary factors on the ability of men to withstand repeated exposures to intense cold. Twelve young

volunteers were subjected to eight-hour exposures in a cold room, either at — 20° F. with considerable protective clothing or at 60° F. with little protective clothing, while they were given diets varying in caloric value and vitamin content. Tests of psychomotor performance and of ability to perform manual operations were made throughout the experiments. The results were largely negative with regard to the questions posed at the beginning of the experiments, but extensive data on the dietary needs of men under these conditions were obtained. For maintenance of body weight, the subjects required a caloric intake exceeding their determined basal expenditure of energy by an average of 93 per cent. There was no evidence that ability to withstand the damaging effects of repeated exposures to cooling environments and to maintain normal neuromuscular and mental efficiency could be appreciably enhanced by giving excessive doses of thiamine, riboflavin, and nicotinic acid above the amount required for normal nutrition. Meals spaced only two hours apart apparently favored the maintenance of body temperature during an eight-hour exposure to cold, as compared with meals given less frequently.

Having in mind conditions faced by the armed forces in arctic regions, three investigators at Cornell University conducted a study of the physiological mechanisms regulating body temperature in men living in an extremely cold environment. The study was planned with special reference to factors of heat production, respiratory exchange, and reactions of the body thermostat in relation to exposure to cold, muscular work, and effects of different levels of dietary protein intake. The proposal for the study was based on speculations regarding the old claim of Rubner that a protein-rich diet through the so-called "specific dynamic action" of proteins can aid the regulation of body temperature, and that increased heat production resulting from the stimulus of cold (chemical regulation) may be replaced by heat resulting from protein metabolism, speculations suggesting that the type of rations supplied to men in the arctic regions might affect their resistance to cold and fatigue.

Extensive metabolic studies were made on a group of young men living continuously for long periods of time in an air-conditioned chamber with environmental temperatures of 60 to 0° F. While wearing varying amounts of protective clothing and receiving different diets, varying especially in the protein intake, the subjects did varying amounts of work each day on a treadmill and served as technical assistants. The data thus collected suggested that the ingestion of large amounts of protein in the daily diet does contribute to the maintenance of thermal balance in man during exposure to low air temperatures. The postprandial metabolism of the men in sleeping bags in the cold chamber was found to be consistently higher, with the same bodily protection, when they were on a protein-rich diet than when they were on diets of lower protein content. The evidence suggested that a high content of

protein in the diet increases resistance to cold, and that the protein-rich diet adds something to the physical comfort and thermal balance of man, during exposure to cold, that is not furnished by clothing alone. Some doubt was cast on the validity of Rubner's theory of chemical regulation of body temperature by the fact that significant increases in basal metabolism were observed under the stimulus of cold only when the subjects were tense or shivering. The investigators concluded that the increase of muscular tension, due primarily to motor reflexes on skeletal muscles, accounted for the elevation in metabolic rate during exposure to cold, rather than the stimulus of cold alone.

The relative dietary protein requirements of men in active and sedentary occupations were studied at the Harvard Fatigue Laboratory. The subjects were inhabitants of a conscientious objectors' camp engaged in their normal activities of desk work, kitchen work, farming, and work in the woods. Three groups of 8 men each were maintained for eight weeks on diets varying widely in protein content. A control group took the regular camp diet, which averaged 100 gm. of protein per day; a second group took a low-protein diet, which was adequate in calories but provided only 50 gm. of protein per day, with no meat, eggs, fish, nuts, or cheese and not more than 4 ounces of milk per day; the third group took a diet providing an average of 160 gm. of protein per day. The controls averaged 13 gm. of urinary nitrogen excretion per day, while the group on the low-protein diet averaged 6 to 7 gm. and the third group 23 gm. Observations included tests of physical fitness and chemical examinations of the blood and urine. Within two months no measurable influence, either deleterious or beneficial, could be observed on the physical vigor or efficiency of the men on the low-protein diet or of those on the high-protein diet.

Another study carried out at the Harvard Fatigue Laboratory was designed to ascertain the early effects of variations in dietary vitamin C on the physical well-being and efficiency of manual workers. The studies were concerned especially with questions of physical fitness because of statements in the older literature that lethargy, lassitude, and inefficiency are the earliest symptoms of scurvy (for example, among sailors), far antedating the onset of clinical signs. In a civilian public Service camp for conscientious objectors, experiments were conducted on twenty-four volunteers engaged in a variety of jobs associated with the camp's work schedule, with a daily expenditure of 2400 to 5000 calories, depending on each subject's job. Four groups of men were closely studied on regimes planned to demonstrate effects of total deficiency of vitamin C, the effects of a good normal diet compared with a deficient one, and the effects of a 75-mg. daily supplement to the normal diet.

The evidence thus collected indicated that where the previous diet had been good, total deprivation of vitamin C, but with a diet adequate in all

other nutrients, for two months did not lead to detectable deterioration in physical vigor, inefficiency, or unpleasant symptoms. Such deprivation did, however, lead to minimal changes in the gums and to desaturation as measured by serum and urinary levels of vitamin C and by tolerance tests. Addition of 75 mg. of ascorbic acid a day to the diet totally deficient in vitamin C appeared adequate to maintain or even to increase the body stores of the vitamin in the majority of the men. The same supplements to a good normal diet appeared to be of no detectable benefit over a period of two months so far as concerned well-being, vigor, or efficiency. Such supplements did, however, lead to increased stores of the vitamin in the body.

Investigating the pathology of vitamin C deficiency with the objective of establishing methods for early diagnosis of scurvy in its incipient stages and of determining the physiological significance of mild degrees of the deficiency in young men, investigators at Northwestern University placed 12 volunteer medical students on a basal diet inadequate in vitamin C and containing minimal amounts of vitamin B complex. During seven months 5 of these subjects remained on the deficient diet alone, 5 received the deficient diet plus daily supplements of vitamin B complex, and 2, serving as controls, received the basal diet plus 75 to 100 mg. of ascorbic acid daily. Data obtained by appropriate tests during this period led to the conclusion that a decrease in plasma ascorbic acid level was the best early sign of an inadequate dietary intake. The decrease in plasma level was paralleled by decreased urinary excretion of ascorbic acid. The ascorbic acid content of the leukocyte-platelet layer did not indicate early depletion so well, but furnished valuable information later as to how protracted the period of depletion had been.

Surgical studies were conducted on all depleted subjects and normal controls at the conclusion of the seven-month period. Skin incisions were made and sutured, and biopsy sections of the healing incision were taken for examination at different stages of healing. Satisfactory wound healing did not occur until body levels of vitamin C were restored and the tissues were resaturated with vitamin C. A marked susceptibility to wound infection was found in the vitamin C-depleted subjects. These observations led to a recommendation that when vitamin C-depleted men were wounded, massive doses of 450 to 500 mg. of ascorbic acid per day should be given to facilitate healing.

Another investigator at Northwestern University studied nutritional factors, especially the metabolism of ascorbic acid, in young men exposed to simulated high altitudes in a decompression chamber. Seventeen medical students served as subjects. When they were receiving a daily intake of 140 mg. of ascorbic acid and were exposed during a period of four weeks, three times a week, to a simulated altitude of 35,000 feet while breathing 100 per cent oxygen, a high excretion of ascorbic acid in the urine occurred, but the data

indicated that actual deficiency would be unlikely to develop at this level of
intake. On the other hand, when the subjects were on a daily intake of
91 mg. of ascorbic acid and were exposed to an altitude of 18,000 feet with-
out supplemental oxygen for one hour, three times weekly, a gradual
depletion of ascorbic acid and a lowered concentration of ascorbic acid in the
plasma were observed. The evidence indicated that the depletion resulted
from increased utilization. Repeated exposures to the same altitude without
supplemental oxygen did not affect the urinary output of thiamine, ribo-
flavin, alpha-ketoglutaric acid, pyruvic acid, and acetone bodies. In another
project, included in the program of research in aviation medicine, these
investigations were extended with further studies on the effects of altitude,
anoxia, and so forth on the excretion of electrolytes and 17-ketosteroids.

In another project at Northwestern University, experiments were con-
ducted to evaluate, in young men of military age, physical fitness and work-
ing ability at high altitudes in relation to nutritional factors. Seven volun-
teers were studied during a period of sixteen months with varying dietary
intake and physical activity at ground level and at simulated high altitudes
(15,000 feet) in a decompression chamber. The observations led to the con-
clusion that these men could be maintained for considerable periods of time
on a diet of 3400 calories with a daily average intake of 0.95 mg. of ribo-
flavin and 0.85 mg. of thiamine without any evidence of physical deteriora-
tion as measured by various types of tests performed at ground level and at
high altitude. It was also shown that physical training at ground level
greatly increased ability to perform work at a high altitude. Valuable bio-
chemical data were collected on the utilization and excretion of the two vita-
mins at different levels of intake.

The significance of corneal vascularity in relation to possible combined
effects of riboflavin deficiency and exposure to strong light was investigated
in two projects, with mainly negative results. Because corneal vascularity
is associated with riboflavin deficiency in experimental animals and in man
and has been observed among men subjected to prolonged exposure to strong
sunlight (for example, shipwrecked sailors and grounded air force per-
sonnel), it was suggested that the two factors might be mutually aggravat-
ing. In experiments conducted on rats at the Public Health Institute of the
City of New York, there was no evidence that strong light decreased the
riboflavin content of the cornea, and no difference was seen in the develop-
ment of corneal vascularization as a result of riboflavin deficiency in rats
exposed or not exposed to strong light. At New York University an inves-
tigator studied the effects of light on corneal vascularization and its relation
to riboflavin deficiency in men maintained on a diet of 1.5 mg. of riboflavin
daily and subjected to varying exposure to sky light and to light from a
carbon arc lamp. There was no evidence of significant changes in corneal
vascularity induced by such exposure.

Investigations conducted by Butler and Gamble in Boston were planned

to gather information for the improvement of emergency lifeboat and life raft rations and information for the instruction of shipwrecked men as to how they might eke out their provisions of water and food until rescued. This study was made under a premise that, aside from accident and exposure, thirst with dehydration is the major threat to the survival of castaways, and that the primary prerequisite of a life raft ration is the defense of body water. Extensive metabolic studies were carried out on volunteer subjects under observation in the laboratory and on rafts at the seashore, under regimes involving varying degrees of deprivation of water and food. The experiments yielded extremely valuable information on the physiological mechanisms that govern water exchanges of the body under varying conditions, and on renal secretion with respect to the ability of the kidneys to concentrate obligatory waste products of the body in a minimal volume of urine during severe water deprivation. The data collected indicated that a castaway ingesting 100 gm. of carbohydrate per day sustains a daily basal renal water loss of about 300 cc., plus a basal extrarenal water loss of 800 cc. Since approximately 300 cc. of water is made available by oxidation of body tissue and excretion of body solutes, the minimal daily water requirement is around 800 cc. The ingestion of 100 gm. of carbohydrate per day provides physiological benefits and conserves body water at least to the amount it would displace water in the limited ration. Additional food in the form of carbohydrate in excess of 100 gm. per day or any fat or protein added to the ration at the expense of water was found undesirable because it increased dehydration by limiting the water intake, reducing the water of oxidation from readily available reserves of body fat, and, in the case of protein, increasing the urinary water requirement.

The conclusion was drawn that the provision of 700 cc. of water and 100 gm. of carbohydrate per day should prevent significant dehydration provided the basal rate of water expenditure is not exceeded. Because extrarenal water loss under optimal conditions is double renal water loss, exposure to sun even in temperate climate and protection from breeze may augment extrarenal loss to several liters per day. Limitation of extrarenal loss is therefore a matter of even greater concern than provision of minimal water intake. If storage of fresh water from intermittent rain is possible, gradual replenishment of water deficit is more beneficial than abrupt replenishment. The addition of sea water to such rain water in the ratio of 1:4, provided no more than 250 cc. of sea water is ingested daily, may increase the effectiveness of such water in replenishing water deficit.

The studies on the possible utilization of sea water by castaways tended to disprove the traditional belief in its toxicity. The ability of the normal human kidney to concentrate chloride was not a limiting factor in the utilization of such amounts of sea water as do not exceed the limit of gastrointestinal tolerance. Since this limit approximates 500 cc. per day, and since a person can obtain for bodily requirements other than urinary excretion

no more than one fourth the amount of sea water ingested, maintenance of body water requirement by sea water alone is impossible. Augmentation of a limited supply of fresh water by one fourth as much sea water, not to exceed 250 cc. of sea water per day, had beneficial effects in increasing the ability to ingest food by augmenting the fluid intake, improving water balance, aiding renal excretion of catabolites by increasing urine volume, and relieving the gastrointestinal discomfort sometimes suffered when life raft rations are consumed with a scanty water supply. However, improvement in the water balance by the ingestion of tolerated amounts of sea water was accompanied not only by an improvement in sodium chloride and extracellular fluid balance but also by a positive magnesium balance and an increased excretion of potassium, nitrogen, organic acids, and intracellular fluid. Because the physiological significance of these alterations in balance could not be appraised, the investigators concluded that the studies did not give a definitive answer to the desirability of castaways' ingesting limited amounts of sea water.

CHEMICAL STUDIES ON VITAMINS

Folic acid (also known as vitamin B_c, *Streptococcus lactis,* and R factor) was discovered to be a factor required for growth by certain bacteria. Then this substance was found in the early war period to be useful in the production of tetanus toxoid and in certain microbiologic assay methods developed for the determination of other vitamin factors. In order to make an adequate supply of folic acid available for assay purposes and for various lines of basic research, R. J. Williams, of the University of Texas, undertook to prepare large quantities of the material. During a two-year period he supplied material to two hundred and fourteen different laboratories, thus greatly aiding work that has culminated in rapid advances in knowledge of this important accessory food factor.

Research on the chemistry of vitamin A was undertaken at Mount Sinai Hospital, New York City, at a time when it appeared that conditions of the war might lead to a serious scarcity of foods containing the natural vitamin, making desirable a method for its artificial synthesis. Work on this project was stopped when the danger of a critical wartime shortage of the vitamin from natural sources seemed past, but before termination of the study much information on the chemical nature of vitamin A and related organic compounds had been gathered and some progress had been made toward the development of a technically practical method of synthesis.

The experiments in many projects of the nutrition research program placed new emphasis on several facts of importance for future research. It is clear that many nutritional questions can be answered only by realistic controlled

experiments with human subjects, and that any proper study of nutrition in man requires a team of experts abundantly supplied with special facilities and equipment. Wartime exigencies forced a realization of the limitations of research conceived on the theory of strict separation of variables, and of the necessity for simultaneous studies of many functions of the whole organism in the development of a true science of human physiological hygiene.

Evaluating the relative success of the OSRD(CMR) program of nutrition research, certain advisors have expressed the opinion that some degree of failure of full fruition and effective application of tangible results of this program came from too great a division of responsibility, leading to actions and recommendations that, through being not always in full accord, if not actually in conflict, failed to find general acceptance in practice. To some extent this development resulted from disagreement or misunderstanding among different groups; in part it resulted from actions of the armed forces and other governmental agencies in taking their problems and requests for advice separately to a large number of advisory authorities, often without adequate efforts at correlation. Some confusion was caused at times by partly duplicated recommendations differing in minor details. The research program did, however, produce an enormous amount of new scientific information, which, aside from its immediate practical value, forms a firm basis for advancing research to be continued in peacetime.

ACCLIMATIZATION TO HEAT AND COLD

EDWARD F. ADOLPH

Mᴀɴ'ꜱ RELATIONS to climate are greatly exaggerated in time of war. Archibald MacLeish tersely stated, "In this war men die by cold, heat, and thirst." Instead of a house, man has a vehicle or a foxhole in which to live. Instead of staying in the locality in which he was trained, he is transported to the desert, the tropics, or the Arctic. He has no time to learn how to combat the extremes; he can only be briefly instructed and given what forms of protection are available. For the rest, he must trust to his own fortitude and ingenuity.

Before protective clothing, shelter, and food can be supplied intelligently, the man himself must be studied. At the beginning of the war, much general information was available, but it was not known specifically how resistance to heat could be increased most quickly, what the human limits were for withstanding cold, or how much shortage of body water could be endured.

Certain problems concerning the effects of heat were already under study before the war. It was known that men could survive any warm outdoor climate on the face of the earth when at rest, but not whether they could work in all climates; and new problems were being encountered with almost every new type of vehicle, ship, or airplane. Battle tanks might be perfect from the standpoints of engineering and armaments, but if troops could not operate them in extreme temperatures, they would fail to be serviceable in some theaters of war.

In 1941, two kinds of warfare were most prominent from the point of view of climate, that in the air and that in the desert. Desert warfare exposed men to heat and to lack of water; aerial warfare exposed them to cold, to a castaway's existence on life rafts, and to other hardships. This was the background that induced several teams of investigators to discover the deleterious effects of exposure to heat and cold and of lack of water and salt.

ACCLIMATIZATION TO HEAT

Work along these lines sponsored by the Committee on Medical Research was begun in the Fatigue Laboratory of Harvard University in February 1942. This was the laboratory best prepared to apply the knowledge of en-
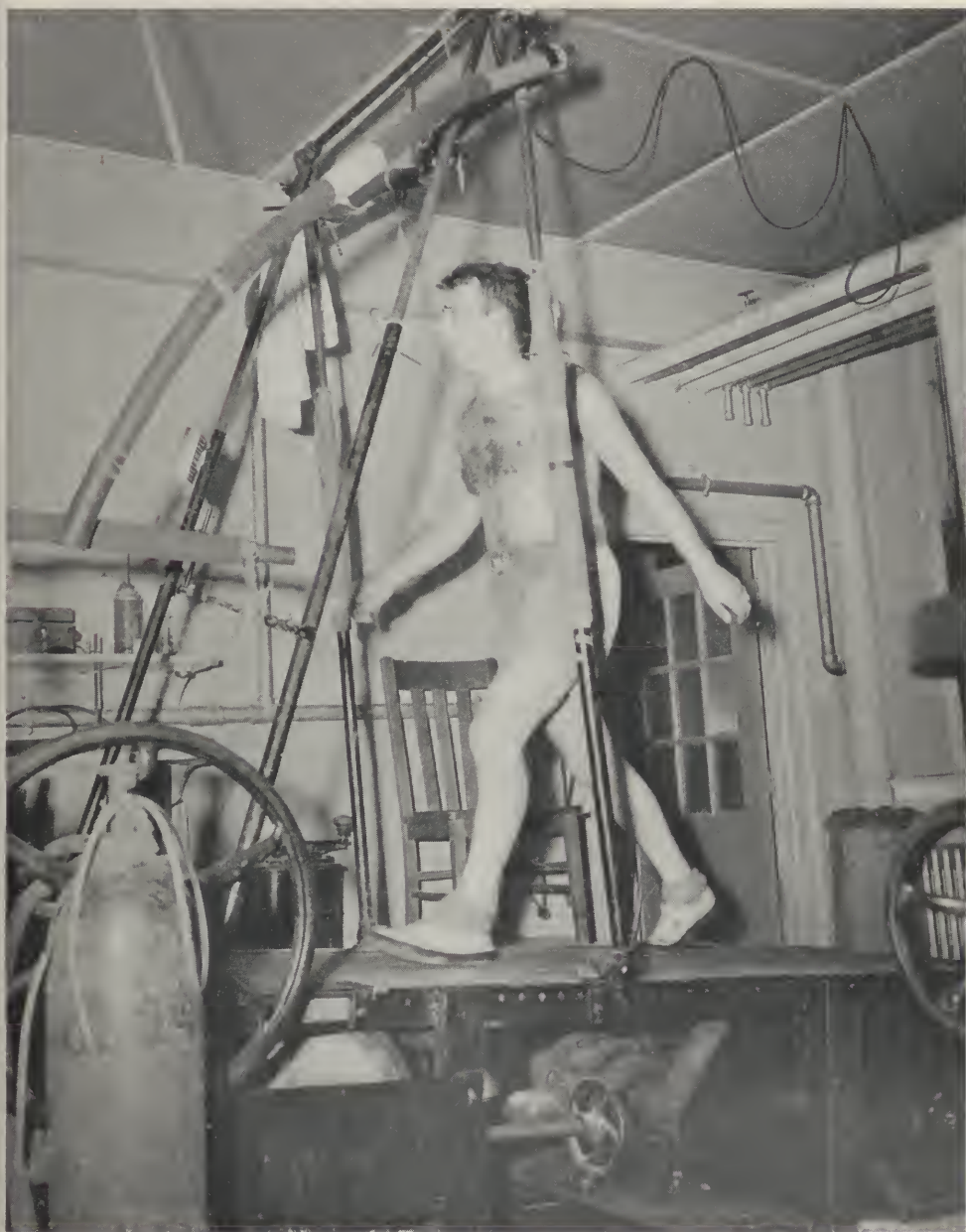
FIGURE 63. *Soldier walking on treadmill in the Fatigue Laboratory.*
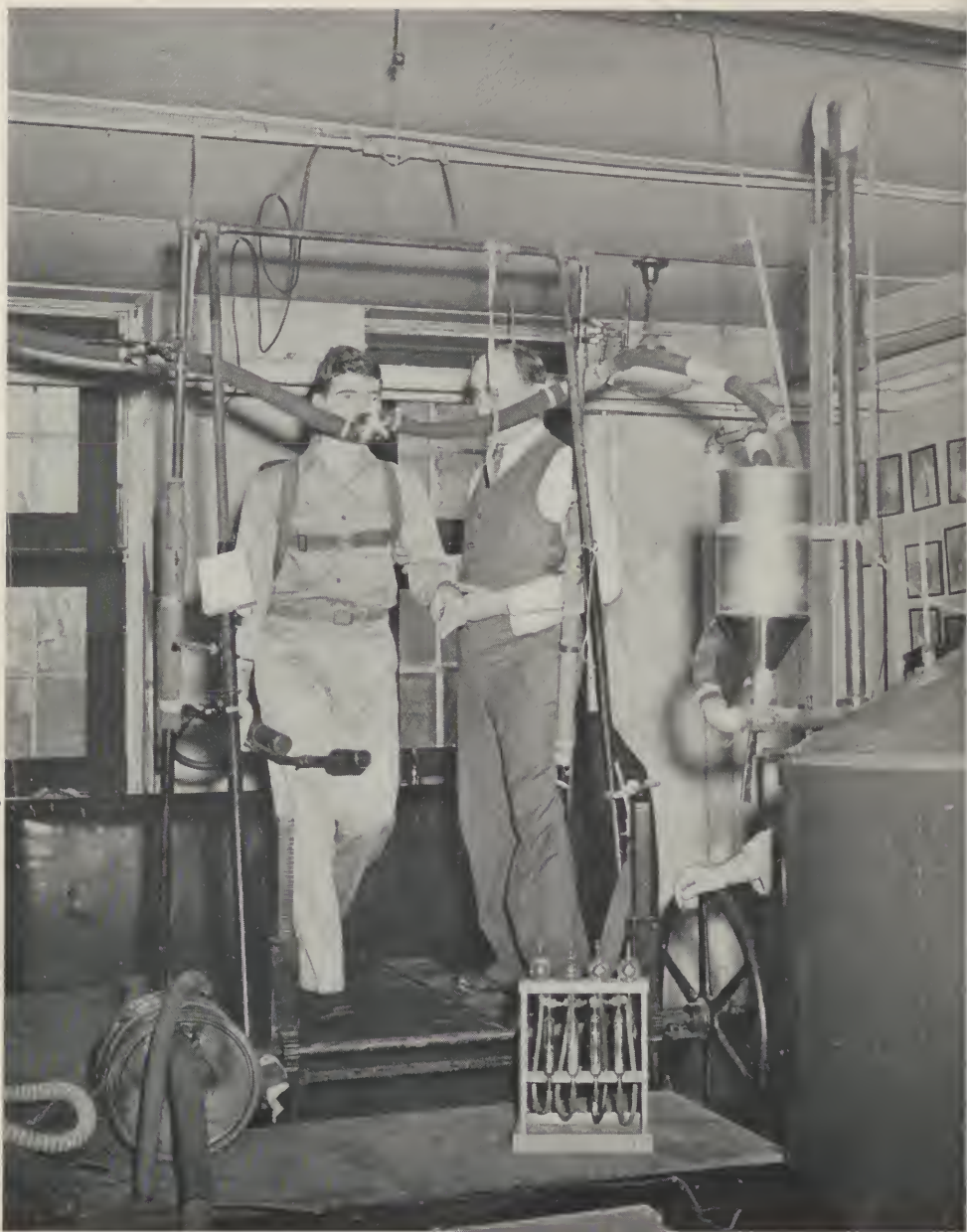
FIGURE 64. *Collecting expired air from a walking man and counting his pulse rate.*

vironmental effects on man, for its investigators had accumulated previous interest and experience in them. Acclimatization to heat, which was already known to exist, was demonstrated to require only a few brief exposures of men engaged in such activities as walking (Fig. 63). It was judged success-ful when they could walk rapidly with minimal increases of pulse rate and rectal temperature (Fig. 64), and apparently those who could not acclima-tize quickly were few.

By the summer of 1942, the need became evident for much wider knowl-edge concerning ability to work in hot surroundings. The chief military campaigns were in northern Africa and in tropical areas of the Pacific. Training centers were chiefly in the Southern States, and troops were maneuvering in the desert areas of southern California. These factors fur-nished the impetus that brought investigators in five universities into the exploration of heat effects. At about the same time, parallel programs of research were under way in the several medical research laboratories of the Army and Navy.

The studies initiated included the bearings of many influences on the response to heat. Physical fitness, diverse processes of acclimatization, nutri-tional status, and requirements for water and salt were represented. Each of these aspects will now be discussed.

PHYSICAL FITNESS

The ability to perform tasks requiring muscular work was thought to be related to heat tolerance, because both heat and work have similar influ-ences on the circulation of the blood. Short tests both with and without exposure to heat were essential for assessment of tolerance. Some of these tests were aimed at measuring the strain on the circulation; this was accom-plished by placing subjects under a severe stress, such as stepping up and down a standard height at a standard pace or tilting in the relaxed state, and noting the rise in heart rate and the fall during recovery. Other tests were aimed at measuring psychomotor and sensory performances, as by pursuit meters, aiming tests, visual field tests, and other methods. The results of these tests were then correlated with ability to perform prolonged work in the heat. This sort of activity included treadmill walking or run-ning, stationary bicycle riding, and outdoor walking. Although each test yielded information that was useful for some particular project, no agree-ment was reached concerning which tests might be considered standard for all purposes.

Evidence was found that ability to work in hot climates without undue cir-culatory strain could be measured by fitness tests. Hence, these tests could be used to screen out unfit persons from among those given a chance to acclima-tize to heat. It was recognized that the strain due to heat was in large part

circulatory. No evidence, however, was uncovered indicating that tolerance to heat could be generally detected without exposure to heat, nor was any adequate search made for such a method.

Acclimatization

Adjustment to working in hot atmospheres was studied both indoors and outdoors. As previously recognized, most physiological changes occurred in the first few days of exposure; these included decreases in pulse rate, rectal temperature, and salt loss, with increases in the rate of sweating and the amount of work performed. Most of the easily measurable acclimatization could be produced by exposing men at work to moist or dry heat only one or two hours a day for four days. Typical atmospheric conditions used in such exposures were 120° F. temperature with 30 per cent humidity and 95° F. temperature with 80 per cent humidity. Acclimatization so acquired persisted for at least three weeks after the last exposure. Hence, men conditioned to life in the desert in summer could be transported long distances without losing much of their fitness. Nevertheless, it was considered advisable that physical work be continued during the interval of transfer.

But acclimatization probably involves much more than the changes enumerated. There is evidence both in animals and in man that profound metabolic modifications supervene. During the days before acclimatization is complete, nitrogen is lost from the body proteins, regardless of the composition of the diet, and sodium chloride is retained. Both sweat and urine are formed with less of these two ions in them. Indication was found that the function of the adrenal cortex is concerned in metabolic acclimatization, for administration of adrenocortical extract shortens the period required for these adjustments. Continued injection of this extract suppresses the hyperfunction of the adrenal cortex induced by exposure of the body to heat, so that when the injections are discontinued the processes characterizing incomplete acclimatization partially recur.

Probably these events are only a part of the intricate series of shifts in many functions that intervene when men are exposed to heat. Somehow the net results of acclimatization are an increased tolerance to heat and, incidentally, a sparing of protein and salt to the body.

Diet

The nutritional status of men in hot climates has been chiefly concerned with the many substances suspected of being differently metabolized in the heat than in temperate climates. The losses of these substances in sweat and urine were therefore studied, and effects of compensating for their loss by adding them to the diet were sought. Prolonged studies were needed to prove finally that losses of vitamins in sweat are negligible, at least for vitamins B_1, B_2, B_c, Bx, C, F_1, F_2, and H, niacin, choline, and inositol. The

evidence regarding vitamin C was especially difficult of interpretation because this vitamin is rapidly destroyed in the sweat itself. Some of the vitamins appeared in sweat in proportion to the amounts ingested, but the total amounts lost in sweat were consistently less than the customary intakes. Similarly, the diversion of calcium, iodide, and iron into sweat was negligible.

Supplements to diet did not help men in hot climates, except for water and salt. At one time or another, oversupply of each vitamin, beginning with vitamin C, was expected to enhance resistance, but in each case careful study led to disappointment. Similarly, supplements of glucose and of protein were shown to have no effect on tolerance to heat and to work. Hence, of all the materials that might be of specific benefit in hot climates, only water, salt, and adrenocortical extract are of known value, and of these water alone is surely needed in larger amounts in hot climates than in temperate ones.

Tolerance

Tolerance to heat could now be defined for acclimatized men at specified activities. In general, these men could do any ordinary work for four hours in a wet-bulb temperature up to 88° F. Slightly hotter conditions might be endured by removing clothing and securing adequate sleep in cooler quarters. On the other hand, tolerance was greatly diminished by radiation, heavier work, ingestion of alcohol, lack of water and salt, wounds, and infections. Within the tolerance limits, the heat imposed serious difficulties that could be measured in terms of higher pulse rates, higher sweating rates, and higher rectal and surface temperatures. High performance in continuous tasks of many sorts could be expected only in the most favorable environments, no matter how strong was the motivation to perform these tasks.

During steady work on a treadmill the rate of sweating decreased after three to six hours. In dry heat the output of sweat was likely to become inadequate for body cooling and thus to limit endurance; in moist heat the diminution of sweating merely conserved water. More often endurance was limited not by inadequate sweat production but by circulatory inadequacy.

Prolonged exposure to heat (day and night for two or three months at 86° F.) in laboratories has been found to produce no physiological deterioration. Persistent reports from tropical countries, on the other hand, suggest profound deleterious effects. The question which of these conclusions is correct requires for its answer careful study by a long-term experiment in a tropical area, under as satisfactory conditions as possible. Until such an experiment is done, the applicability to tropical environments of all the newer knowledge of heat effects will not have been established.

Water Loss

Water has been found to be of the first importance to men working in the heat. Sweat may be lost in the desert at rates up to 1.7 l. per hour, and in

the tropics up to twice this rate. Its output in given activity is proportional to air temperature above 90° F. Up to 11 l. of sweat may be formed in a day. When the fluid so lost is not completely replaced by drinking, physical disability results. This disability appears to stem from the diminution in volume of blood that is available for circulation. It takes the form of increased rectal temperature and pulse rate, decreased capacity for muscular work, dizziness, nausea, and impaired judgment. This syndrome constitutes dehydration exhaustion.

The widespread notion that men can be conditioned to tolerate dehydration turned out to be incorrect. Sweating is about as rapid during dehydration as during superhydration, its rate being dictated by the excess of heat present in the body. The dehydrated person becomes sensitive to overheating in proportion as his circulation becomes inadequate. Hence, requirements for water can be diminished only by reducing the gains of heat by the body — from work, from sunshine, and from air hotter than the skin. Experience and training help to conserve water only by teaching men how to reduce exposure to heat.

In the desert a man is incapacitated for work when he has lost 4 to 5 kg. of water from his body. At an 8-kg. deficit he can do nothing but lie still. He may survive a deficit of 12 to 15 kg. if he can keep cool. On a life raft, the water loss can be slowed by protection against the sun and by using sea water for cooling in the place of sweat. In this event it may take a week or two to lose 15 kg. instead of a day or two as on the desert. Prolonged inanition makes it possible to survive with less water but of course lowers ability to work.

Neither the sensation of thirst nor the physiological effects of dehydration can be avoided without a supply of water. Drugs and special food substances are without avail.

From studies made on soldiers in the desert and on life rafts in the tropics it is possible to relate the amounts of water needed to environments and activities. The amounts required for optimal physiological performance exactly equal those being lost by sweating. Actually the urge to drink lags behind the need for enough water to improve the circulatory status. Once the amounts required have been related to the environmental temperatures, it is possible to construct for all desert lands and all warm oceans maps that indicate necessary water supply and possible length of survival without water.

SALT LOSS

Salt (sodium chloride) is the chief constituent of sweat, but acclimatized men may have less than 0.03 per cent of it present there. With the highest rates of sweating, therefore, only 3 gm. per day need be lost by this pathway in most men. There may be some persons who cannot form such dilute

sweat and are therefore dependent on high intakes for preservation of the bodily content of salt.

Some evidence has been reported that heat exhaustion occurs with special frequency in men on a low intake of salt. Heat cramp occurs under similar circumstances and is specifically relieved by administration of salt. No disadvantage of excessive salt intake is known, so long as plenty of water is available with it, and for this reason investigators have felt safe in recommending to all men exposed to high temperatures a plentiful intake of salt in dissolved form. At present there is no guide to salt intake other than the principle of making it plentiful. Much is yet to be learned of the role that salt plays in metabolism, sweat formation, and maintenance of the circulation. Without this knowledge there is no way of visualizing how the body's salt content can be of prime importance to some men working in the heat.

ACCLIMATIZATION TO COLD

Exposure to cold was systematically studied relatively late in the war. The problem of the general effects of chilling, as contrasted with the local exposures that produce frostbite and trench foot, became progressively acute with increased incidence of shipwreck, high-altitude bombing missions, and ground operations in high latitudes.

Attack on this problem during the first year of the war was largely confined to the laboratories of the Army and the Navy. At the Harvard Fatigue Laboratory investigations of clothing were early initiated. In this period, the conviction was reached that improvement of clothing could not alone protect men from cold. Inquiry into acclimatization to cold was undertaken at the University of Illinois. In 1944 more prolonged acclimatization was studied at Cornell University. In 1945 a beginning attempt was made at the University of Rochester to define the limits of tolerance to cold.

It may be stated that no one has satisfactorily demonstrated any physiological acclimatization to cold. Such may well exist, but none of the characteristics of heat acclimatization have served to indicate how much the tolerance to cold is increased. Changes in blood volume, thyroid function, adrenocortical function, and other factors have been described, but to what degree these help in improving resistance to cold is unknown. During the war investigators attempted to measure the effects of clothing, diet, and other factors on resistance to cold; they succeeded in comparing rates of body cooling only. Methods of measuring the elementary assessment of resistance are still to be found.

CLOTHING

Nevertheless, the effects of cold were explored. Data were accumulated by the device of comparing the temperatures of chosen points on the body

surface during paired exposures with various protective devices. Numerous articles of clothing, including electrically heated suits, were so tested during diverse activities. One of the points of interest was the difficulty of doing much work in the cold without sweating. Sweating, however, reduced the insulation of the clothing. Although methods were available of inhibiting sweating, both generally and locally, the safest method appeared to be, whenever possible, to reduce the clothing to the point of discomfort before starting any physical work in the cold.

Diet

The role of diet in influencing the rate of body cooling in the cold cannot be a very simple story. The smallness of decrement in rectal temperature and the results of a series of psychomotor tests indicated that during exposures of four to eight hours diets high in fat and carbohydrate induced better tolerance to cold than did diets high in protein. The taking of small meals every two hours was more advantageous than the taking of fewer and larger meals. Vitamin reductions and supplements (vitamins B_1, B_2, and C and niacin) had no marked influence according to these two criteria of resistance, except for a temporary decrease in maximal rapidity in a test of tapping with decrease in the intakes of all four vitamins.

The role of the diet was investigated in a different way by studying men living continuously in a cold room. Temperatures were decreased in steps, and to some extent the men compensated by wearing more clothing. They also chose to exercise more in the lower temperatures. Their metabolic history revealed no change that could be said to constitute acclimatization to cold. Heat production was higher when the diet contained 26 per cent protein than when it contained 12 per cent, and it could only be inferred that increase of this factor helped the men to resist cold.

Tolerance

The most remarkable aspect of the long-term cold experiments was the ability of men to maintain themselves without too much discomfort in conditions that are ordinarily thought to be unbearable. Subjects were able to sit and read in temperatures of 40° F. clothed in woolen suits, provided they exercised every hour or two.

Tolerance to cold in short exposures was studied in somewhat simplified circumstances by exposing men outdoors nearly nude. It was found that the posture (lying, sitting, or standing) had no measurable influence, but sunshine and shade, wind and protection from wind, produced differences of heat exchanges and of shivering that could be evaluated as equivalences of air temperature. It was ascertained that the shivering man had a mean

surface temperature lying between his fully maintained rectal temperature and the particular air temperature to which he was exposed, but nearer the latter. Hence, his deep tissues were insulated more by his outer tissues than by the air around him.

Shivering may be accompanied by a rate of heat production five times the basal rate. This means that shivering is an extremely fatiguing activity and cannot be maintained indefinitely. In extreme exposures men become progressively comatose and unable to act intelligently. This may be a factor that frequently limits their survival.

When men are immersed in cold water, as often occurs after shipwreck or airplane ditching, cooling is especially rapid. Unless a man has a suit that water cannot penetrate, he is entirely dependent on the internal insulation supplied by the outer tissues of his body. In water he can probably survive for eight hours a temperature no lower than 62° F.; in air, even though nude, he can endure a temperature as low as 46° F. for a similar time.

It seems fair to say that during the war useful applications could be made of the understanding of the relations of man to heat, water, and salt, because by 1941 some basic generalizations had been drawn about each one of them. Exploratory work had been done, so that systematic deductions could be drawn from well-founded rules. In contrast, the relations of man to cold are even today known only in outline. No one knows enough about the processes that help to resist cold, and the interrelations of these processes, to demonstrate acclimatization. Multitudes of facts have accumulated, but no key to their co-ordination has been found. This is a field that must be cultivated by investigations of great variety before any but the most empirical applications can be attempted. New concepts need to be tested in the hope of finding some sure ground on which to base future applications. The latter include the design of clothing, shelter, vehicles, ventilation, and heating.

SUMMARY

Man's physiological relations to his environment were investigated during the war in terms of tolerance to heat and cold. Factors influencing tolerance to heat were sought in acclimatization processes, in nutritional measures, and in physical equipment. Acclimatization was accurately measured in modifications of circulation and in metabolism of chloride and nitrogen, but many more constituent processes remain to be explored. Nutritional measures reduced themselves to supplying enough water to replace all that was lost in sweat, and plentiful salt. Unusual supplements of vitamins or of protein were not needed. Physical equipment was shown to be of physiological value only insofar as it reduced the need for sweating and the heat increment to the body.

In the cold, nutritive factors seemed to be of little consequence provided plenty of food was available. Long-term exposures showed that men could remain healthy in uncomfortably cold atmospheres for several months. In exposures lasting for a few hours men shivered and lost stored heat to the point where fatigue set in, at which point their resistance to cold failed. These results are only small items in the story of man's relation to cold atmospheres and to cold immersion, a subject that requires extensive further study.

CHAPTER XXXIII

PROTECTIVE CLOTHING [1]

SID ROBINSON AND H. S. BELDING

O PERATIONS in World War II were carried out in all climates of the world; climatic stresses ranged from the hot, dry desert and humid tropics to the cold, windy Aleutian Islands and the frigid temperatures of the subarctic. Superimposed on the various environmental stresses to which troops were exposed was the necessity for performing a wide variety of activities. Aerial gunners exposed to extreme cold had to remain inactive in cramped quarters for hours; soldiers in the steaming tropics often had to continue forced marches for long periods. With aviation playing a major role, many operations involved rapid flights from torrid ground positions into subzero temperatures at high altitudes. In many cases wrecked or damaged planes and surface craft cast men into cold northern waters. Extreme environmental stresses were not, however, always the most difficult ones with which our forces had to deal. For example, the prevention of trench foot in men exposed for long periods to moderately cold climates was one of the most serious medical problems of the war.

In order to carry out operations successfully under these various stresses men had to maintain optimal efficiency and morale, and proper clothing for each situation was certainly a major factor. For this reason, the Committee on Medical Research established a number of projects for attacking the clothing problem from both the physical and the physiological aspect.

COLD–WEATHER CLOTHING

The literature of arctic exploration is full of worth-while advice on how to keep warm in extremely cold weather. The methods proposed all involve properly balancing clothing, shelter, and bodily activity to maintain comfort under prevailing conditions of temperature and wind. From the practical

---

[1] The authors of this chapter wish to express their appreciation to the numerous workers in the field who provided reports of their work and particularly to Doctors H. C. Bazett, L. H. Newburgh, and Lyman Fourt, who respectively wrote the paragraphs dealing with the work on impermeable barriers, exposure suits, and physical measurements of water vapor transfer through fabrics.

In accordance with the Editorial Committee's policy, names of investigators have been omitted. A complete listing of contracts and a bibliography of papers and books published by investigators will be found at the end of Volume II.

experience of explorers many of the principles governing the selection and use of cold-weather clothing are known. For example, it has been established that in extreme cold no conventional clothing assembly is at the same time warm enough for standing around and sufficiently free of bulk to permit freedom of movement. During sleep, however, when bulk is no disadvantage, adequate protection can be provided. It is also clear from the arctic literature that clothing should be reduced in amount or loosened to avoid sweating during periods of hard work. Even when these precautions are taken, enough moisture accumulates in the outer clothing to make it stiff with ice and considerably reduce the insulation provided.

During World War II this practical experience of explorers was invaluable, but new problems also arose that could not be answered from experience. One such problem was this: with fur clothing unavailable what constitutes the best clothing that can be provided for half a million to a million men to protect them against near-arctic conditions? Another problem was the protection of thousands of men who were to be exposed to extreme cold and high wind while operating machine guns and gun turrets in heavy bombardment aircraft. Certain aspects of both these problems were studied.

Early in 1942, shortly after completion at the Harvard Fatigue Laboratory of a cold chamber designed for operation at temperatures as low as − 40° F., the New England Committee appointed by the Quartermaster General of the Army for the study of winter equipment asked the Laboratory to help in differentiating between the protective values of several types of sleeping bags. To the comments of men exposed in these bags were added objective data on skin and rectal temperatures. It is said that this was the first report received by the Quartermaster General containing objective data obtained on men during actual exposure to extreme cold. The result was a deluge of requests for physiological evaluation of standard and experimental clothing designed for use by troops in extreme cold. Under this pressure new methods for testing were evolved by a team of workers, and soldiers were assigned to temporary duty as technicians and as subjects of experiments.

Methods of Evaluating Clothing

Some of the methods evolved for evaluating clothing have been published in *Clothing Test Methods,* edited by L. H. Newburgh and Milton Harris. For resting men sleeping bags and daytime clothing were evaluated as follows. The heat lost from the body was considered to be the sum of metabolic energy production and loss of body heat due to a fall in temperature of the body mass, as judged from the fall in rectal and mean skin temperatures. The part of this heat that was lost by vaporization of water from the lungs and skin was calculated from measurements of loss in body weight during

the exposure. This was subtracted from the total heat loss, and the remainder was considered to represent the loss of heat through the clothing by convection, radiation, and conduction.

A suggestion that the protection given by clothing be expressed in units of insulation, understandable by both military leaders and laymen, was made by a group of investigators. The unit proposed was the "Clo," one Clo being defined as the protection provided by an ordinary business suit. The insulation value of the experimental clothing was calculated from knowledge of the heat flow through the clothing, the temperature gradient from the skin to the ambient air, and the insulation of the air layer just outside the clothing (the latter being a known function of wind velocity and altitude).

Evaluation of clothing involved more than formal determination of its insulation in that an effort was made to simulate normal conditions of use and to record comments of the wearers. Sleeping gear was used overnight in various degrees of cold, with and without tents, and on various surfaces — wood, cement, boughs, snow, and various types of pads. The clothing for daytime use was worn during and after hard work, on one occasion continuously for four days, in order to determine the effects of sweating and acclimatization on comfort. While most of this work was done in the laboratory, some field experience was accumulated. One of us (S. R.) spent several weeks using experimental clothing under winter field conditions in Alaska.

As a check on the results of experiments on men, several thermostatically controlled, electrically heated copper "men," "hands," and "feet" were constructed. The internal and "skin" temperatures of these devices were kept at a constant level by maintaining a suitable heat input, the skin temperatures being determined by means of thermocouples and the heat input by a sensitive watt-hour meter. This was found to be a particularly good way to determine the insulation provided by assemblies of clothing, and later to study moisture transfer from wetted underclothing through the outer clothing.

SLEEPING BAGS

Probably the single most important characteristic of a sleeping bag is a large ratio of insulation to weight. It is also considered desirable for a bag to have small bulk when packed and to be made of materials that have a degree of water-repellency. Much of the difficulty in selection of materials arises from the fact that those best suited from the point of view of insulation and weight are so compressed under the weight of the wearer that much heat is transferred to the ground. This means that with a down-filled bag a good pad is absolutely essential; most such pads weigh at least 2 pounds and are only large enough to reach from the shoulders to the hips.

What kind of bag is needed to provide fairly adequate protection to a

partly dressed man? He will be provided with barely enough insulation at 32° F. with a bag sewed from two layers of Army blanketing and covered with a light cotton windbreak (4 Clos, weight 10 pounds). On the other hand, he will be equally comfortable at zero in a single bag of quilted down and waterfowl feathers (7 Clos) of somewhat less weight if a pad is provided beneath him. For — 40° F. he needs two layers of quilted down and feathers (12 Clos, 16 pounds) with a pad. One of the best bags for its weight is made of reindeer skin; it combines a very thick insulating layer with light weight and high resistance to compression, largely owing to the fact that deer have hollow hairs.

After testing over sixty types of sleeping bags, it was possible to rank materials available to the Army in descending order of efficiency about as follows: (1) 40 per cent down and 60 per cent waterfowl feathers (the down could even be decreased to 20 per cent without any serious reduction in insulation); (2) waterfowl feathers; (3) chopped chicken feathers; (4) turkey feathers; (5) cellulose acetate fibers (⅔ to 1½ deniers); (6) multiple layers of single-faced alpaca pile; (7) multiple layers of double-faced wool pile; (8) wool or kapok batting; (9) wool blanketing. Two materials of special interest were tried. One, milkweed floss, was the best for its weight of any material when new but deteriorated with a few uses in the cold room, probably because the floss is brittle. The other was cellulose acetate ("Bubble-fil") consisting of chains of small, air-filled beads; this gave splendid insulation, but the bags were extremely bulky when stored.

The size of a sleeping bag is a matter of some importance. A given weight of filler, say 6 pounds, is more effective in a closely fitting "mummy case" bag than in a roomy bag because less surface is presented for heat loss and because with reduced area the bag can be thicker. On the other hand, a bag that fits too tightly not only may give rise to claustrophobia but may actually give a big man reduced protection because of compression of the filler from within; for this reason, little or no advantage is obtained from squeezing into the present Army Mountain Bag while fully dressed.

CLOTHING FOR ARCTIC AND SUBARCTIC USE

Laboratory tests were made in order to evaluate many of the experimental assemblies of clothing that were developed by the Research and Development Branch of the Office of the Quartermaster General. The first assembly tried for arctic use simulated in design the fur clothing of certain Eskimos. The underclothing consisted of a loosely fitting parka and loose trousers (supported by wide suspenders) of rather dense, medium-staple alpaca pile, with the pile ends directed toward the skin. The next layer consisted of similar garments of long-staple alpaca pile with the ends directed outward. Over this were worn closely woven cotton windbreak gar-

ments. Roominess in the region of the trunk was sufficient to permit withdrawing the arms from the sleeves in against the chest. The outer layer was equipped with drawstrings at the neck, waist, and lower margins of the parka and at the waist and cuffs of the trousers, to permit passage of cold air across the skin while the garment was opened during exercise and a high degree of insulation when it was closed. Handgear consisted of woolen mittens covered with large fur "hand warmers" and footgear of several pairs of heavy wool socks, a pair of felt socks, and canvas "mukluks" with flexible leather soles. The flexibility and loose fit of the mukluk combination allows free circulation in the feet and is necessary in unheated footwear for subzero environments. A stiff leather or rubber shoe is dangerous even though it may be large.

This arctic outfit provided the wearer with between 4 and 5 Clos of insulation and represented the practical upper limit of protection for daytime clothing. At zero a man could sit comfortably for three hours without undue chilling; at − 40° F., for an hour.

In this outfit, as in all others studied, the hands and feet became cold before other parts of the body. There are internal and external reasons for this. There is little active tissue in these members, and circulation through them is slow (probably as little as 1 cc. of blood per 100 gm. of tissue per minute during vasoconstriction from exposure to cold), with the result that their cooling is practically a function of their specific heat and mass. Added to this is the fact that the radius of curvature of these members is so small that successive additions of hand and foot gear increase the surface for heat loss almost as fast as insulation is added in the form of thickness. The net result is that impossibly large gear would be necessary to provide adequate protection for the hands and feet of a resting man, even at 10° F. Fortunately, during exercise in adequate body clothing circulation to the hands and feet is increased so markedly that they may readily be rewarmed after cooling.

Although the particular arctic assembly described above was never adopted as such by the armed services, some of its principles were. Pile clothing was widely used in jackets and parkas; outer clothing was always closely woven to break the wind; mukluk assemblies were provided for use at arctic bases; and the so-called "layering principle" was adopted, by which protection could be suited to prevailing conditions by putting on or taking off sweaters, jackets, and undertrousers.

Electrically Heated Clothing

With the development of the Flying Fortress as an offensive weapon, the Army Air Forces were confronted with the problem of protecting relatively inactive men against severe wind blast at temperatures as low as − 60° F.

To answer the problem an electrically heated suit was developed that drew about 350 watts, distributed between a coverall, heated slippers, and heated gloves. In 1942 these garments were used in laboratory experiments in connection with high-altitude chamber studies in extreme cold. Electrical failures of the gloves and slippers were numerous, and even when failure did not occur the distribution of heat in the garments was unsatisfactory. Consequently, early in 1943 a laboratory project was initiated to determine the principles involved in providing protection with electrical heat. Experiments showed that heat is effective for body warming in proportion to the fraction of total insulation lying outside the wires; for example, if three quarters of the total protection provided in the absence of heat lay outside the wired layer and one quarter inside, applied heat was 75 per cent effective for heating the body and 25 per cent effective for heating the ambient air. These results had previously been predicted, and an ingenious nomogram had been devised by an electrical company to indicate heat requirements of clothing as a function of positioning of wires, ambient temperature, and total insulation worn. In July 1943, the Army Air Forces made a request for help in the development of more satisfactory heated flying clothing, and particularly the specification of heat distributions and amounts that would give balanced and adequate protection under the severest conditions likely to be encountered.

The F–3 heated suit, the last one issued by the Army Air Forces during the war, incorporated features recommended jointly by two laboratories engaged in this field of investigation. Six heated items were included in the assembly. The jacket and trousers were composed of two layers of rayon with wires sewed between them; the former was short and resembled a mess jacket; the latter was cut like a pair of overalls, with heat provided to the chest and back by means of high "bibs." With such an assembly a minimum of sizes was needed. Other garments included such basic uniform items as long underwear, wool shirt, and trousers, all worn underneath the heated garments, and a pile-lined, windproof jacket and trousers, worn outside.

The hands were protected by thin nylon gloves, which were covered by leather heated gloves with woolen linings. Even the latest production models of these gloves were rather poor from the point of view of heat distribution and flexibility, although much effort had gone into glove development. The difficulty was that distribution of heat to the different parts of the hands and fingers is critical. It was shown that properly a large amount of wire should be used and that there should be special provision for altering the heat supply to the palmar surface when materials with different heat conductivities were grasped. An ingenious method was devised of weaving insulated tinsel wire into knit gloves so that it was not felt or seen, but this was not considered feasible for quantity production.

During most of the war the feet had been protected by heated felt slippers that fitted over woolen socks and under a shearling-lined rubber-and-leather boot. However, after escaped fliers who had been downed in enemy country reported that they could not walk with the complete foot assembly because it was too heavy, or without it because the felt slippers gave no support and were soon worn out, a removable heated liner was developed for the shearling boots, and these boots were redesigned to fit over ordinary Army shoes. With this assembly men were enabled to walk away in ordinary footwear after discarding their heavy overboots.

The complete F–3 assembly gives about 3 Clos of protection when unheated, and about 8 Clos when supplied with 250 watts, its nominal power consumption. Such protection will provide comfort for air-crew personnel indefinitely at − 50° F.

Assistance was also given the Army Air Forces in developing a heated casualty blanket for emergency use in heavy bombardment aircraft. These blankets were designed to compensate automatically for changes in ambient temperature.

RADIATION BARRIERS

Radiation may account for up to 30 per cent of the heat lost through clothing. To the extent that this loss could be blocked by infrared reflectors the protective value of clothing would be increased. Two groups of investigators have worked on this problem. Studies made of a variety of woven metallic threads or ribbons showed that reflectivities above 55 per cent are difficult to obtain. The best fabric tested by either group was developed at the Textile Foundation Laboratories with the co-operation of industry. This was an acetate rayon on which vaporized aluminum had been condensed from a vacuum. Reflectivity up to 90 per cent of infrared rays was obtained when this fabric was worn with open-knit spacers inside it. Such insulating systems appear to be advantageous from the point of view of insulation combined with lightness over the lower range of insulation values, but not when a large amount of insulation is necessary.

The results of experiments with reflecting layers in glove assemblies were reported. A hand calorimeter indicated only a small advantage in a glove made up with two opposing reflecting layers of aluminized cloth with a string separator. When this arrangement was used over a heated glove, the heat needed to maintain a steady and satisfactory hand temperature was 10 to 15 per cent less than that required without the reflector. All the workers engaged in these experiments have concluded that at present unsolved problems of durability and production make the extra insulation obtainable from reflecting barriers in cold-weather clothing impractical.

MOISTURE IN CLOTHING

Several considerations led to the investigation by a laboratory group of sweating and of the fate of sweat when men are working in the cold. The first of these considerations was the observation that mukluk assemblies worn during moderate work picked up over 200 gm. of moisture (about one fourth of their dry weight) during sixteen hours of use and that very little moisture was to be found in the innermost socks. The second was the observation during a continuous four-day exposure of men at 0° F. that a regular morning bout of exercise resulted in thorough wetting of the underclothing, but that during the rest of the day and night most of the moisture left the underwear and appeared in the outer clothing; the latter steadily picked up moisture during the entire four days. The third consideration was the result of a search of the literature on arctic exploration, which indicated that the moisture taken up by clothing used on the trail presents a real handicap.

Even after an extensive study, several aspects of this complex problem are still incompletely understood, but certain generalizations may be made. When men sweat as a result of heavy work in cold weather they are under heat stress, with the same manifestations of elevated internal temperatures and pulse rates as those exhibited by men in warm environments, except that active sweating may occur when the mean skin temperature is well below 90° F. By exposing men in very inadequate clothing during hard work (six times the basal level) active sweating may be almost completely inhibited and internal temperatures may be kept from rising (they may actually fall slightly); under these circumstances the mean skin temperature falls to 70° F. or below. However, it is impractical to underdress men during hard work in the cold to the extent necessary to bring the rate of sweating below 100 to 200 gm. per hour.

Most of the sweat produced when men are heavily dressed is retained in the clothing. If sweating is moderate the water is transferred as vapor from the skin to the outer clothing, where it condenses because the vapor-holding capacity of the cool air in these layers is so small. If sweat production is heavy the underwear soaks up and holds much moisture; this is a potential source of evaporative heat loss from the skin during later periods when the body is inactive and when conservation of heat is a problem. Sweat secreted during work is inefficient for body cooling during the work insofar as it is not evaporated, and is partially ineffective when evaporated at the skin and later recondensed in the clothing: in fact, calculations have shown a net efficiency of sweat for body cooling of only 40 to 60 per cent under many conditions. This means that sweat production is usually double what it would be under equivalent conditions of moderate heat stress when men are nude.

For men who remain on the trail under frigid conditions, sweat is a nuisance and a hazard that cannot be completely avoided, because some sweating always occurs. It continues to accumulate in the outer clothing and in the sleeping bag, reducing insulation by replacing dead air space with ice and water and causing the fabrics to stiffen like sheets of armor plate. Lacking a warm shelter for periodic drying of clothes, about the best that can be done is to control work output so that it seldom exceeds a moderate level and to underdress to the point of feeling cool during work. Under such conditions the rate of sweating will be as low as 100 to 250 gm. per hour.

The Brynje system, designed for use in moderate cold, and enthusiastically supported by users as a method of reducing sweating during work and increasing protection during rest, was given laboratory investigation. This system incorporates a loosely knit vest of coarse string, which is worn next to the skin and over which a shirt, sweater, and windbreak garment may be worn. It is claimed that when the outer clothing is loosened there is a chimney effect through the vest and sweating is considerably reduced, and men feel less warm during work. On the other hand, when they rest, closure of the outer clothing prevents movement of air through the vest and increases the effective thickness of immobilized air. In experiments in which sweating, skin temperature, and comfort were determined, the subjects slightly preferred the Brynje system, but no clear-cut objective evidence in its favor was found. This system should be re-examined more carefully under the methods now available for partitioning heat loss.

Clothing that has been thoroughly dried before use absorbs considerable moisture when taken into the cold. The heat given off in the condensation and adsorption of environmental moisture may actually make a small contribution to body warmth. This situation was taken advantage of, probably unwittingly, by the Army Air Forces when it provided drying rooms for flying clothing. A study of the moisture-adsorption properties of textile yarns at low temperatures indicated that between -20 and $+40°$ F. adsorption by wool may be 25 per cent of dry weight and that by cotton 13 per cent.

IMPERMEABLE BARRIERS

The question arose whether the heat loss from the hands or feet could be effectively reduced by preventing evaporation of sweat. It was determined that when light synthetic-rubber gloves were worn under and over a simple woolen glove, the protection appeared to be somewhat superior to that given by a heavy wool-and-leather combination with no moisture barriers. The inner rubber glove was used to prevent evaporative transfer of the moisture from the skin, while the outer glove was used to prevent wetting

of the wool by environmental moisture and as a windbreak. When such an impermeable combination was tried in the field in Newfoundland, it was found impractical because of the moisture that collected within the inner glove. When the combination had to be removed in the cold this moisture sometimes froze in the glove, and in any event made its replacement on the hand a difficult and unpleasantly cold task.

A similar system of impermeable layers was then tried on the foot. In this case a thin cotton sock was usually worn next to the skin, over this a rubber sock or one of some other material impermeable to both water and water vapor, over this again a heavy woolen sock, and over all a second rubber or plastic sock. Again the thick insulating sock was protected from wetting from both sides. When this assembly was tested in Newfoundland at near-freezing temperatures, it was found that the inner cotton sock became moist but that the collection of moisture was slight and negligible for exposures lasting a day or so. Such a combination provided excellent protection against wet cold and allowed men to walk through the snow and ice-water streams all day with impunity. Since the wet inner sock did not have to be removed and replaced, no trouble was experienced.

Laboratory experiments demonstrated that a bare foot kept in practically still air at an ambient temperature slightly above freezing had a total effective protection against heat loss through all channels, including evaporation, equivalent to 0.52 Clo. Under these conditions foot cooling was rapid, averaging 0.01° C. per minute per degree of difference in temperature. When a foot was covered by a completely wet service boot and sock, effective insulation was no greater because evaporative heat loss was enormously increased, but the foot did not cool so rapidly because of the added effective heat capacity of the water in the assembly. A rubber sock inserted between the wool sock and the boot had little beneficial effect when all three were wet, because the water could still be evaporated at the skin and transmit heat as it condensed on the inside of the cooler rubber sock. On the other hand, the effective insulation obtained with the combination of a wet boot, rubber sock, and dry woolen sock was practically identical with that obtained with a completely dry assembly (about 1 Clo); the heat lost by vaporization of water from the boot was taken almost entirely from the surrounding air rather than from the foot itself. It was also shown that the insulating value of the woolen sock is little impaired by the moisture derived from the skin under cold conditions except after many hours.

Since rate of cooling with a dry assembly was no slower than with a wet one (because of the extra heat capacity of the water), it was concluded that times of tolerance to cold can provide no real measure of relative insulation values unless the effective heat capacities of the assemblies being compared are the same.

·

HOT-WEATHER CLOTHING

The combat soldier who wants to be well dressed in the tropics cannot, unfortunately, affect the spotless white linen suits that Hollywood has made traditional for dwellers in tropical regions. The soldier has problems of uniform, laundry, camouflage, and so forth, as well as the physiological problems of coolness, warmth, and protection against insects and trauma. The tropical soldier's clothing must be cool; in its capacities as a reflector and insulator it protects the body to some extent against absorption of heat from an environment with high air temperatures and intense radiation, but at the same time it acts as a barrier to the dissipation of body heat, a factor that is particularly important when men are working. Keeping the clothing clean and dry is a vital factor in skin health, the maintenance of which is now recognized as the most difficult medical problem of troops in the tropics. The soldier has to spend his nights with only his clothing as protection from cold and insects. In wet tropics he is exposed to rain and must frequently wade through mud or water. Therefore his clothing should retain a minimum of water when wet and should dry quickly. The latter properties contribute much to his warmth and comfort when he is exposed at night, since the heat of vaporization involved in drying his wet clothing is extracted from his resting body. He may have to go for weeks without replacement of clothing, so that its durability is a major concern.

PHYSIOLOGICAL METHODS OF TESTING

The strain shown by a man exposed to hot environments may be indicated by his rate of sweating, skin temperature, rectal temperature, and heart rate. Variations in the clothing he wears may seriously affect the degree of heat strain that he will exhibit under a constant environmental stress. Of the above measurements, the rate of sweating and the skin temperature are most sensitive to changes in heat stress or clothing. The heart rate and rectal temperature are insensitive measures in conditions in which the subject is under little heat stress, but become increasingly important measures as the stress is increased. These physiological responses to heat stress exhibit themselves most uniformly if the latter is superimposed on a constant work stress.

In comparisons of clothing, it alone should be varied between experiments, and all other factors influencing the reactions of the subject should be kept as nearly constant as possible. The major factors influencing the heat stress imposed by clothing are as follows: fabric, considered as to the effects of variations in material, thickness, density, air permeability, water-vapor permeability, and water absorption; design; ventilation; impregnites; and fabric dyes. Other such factors are air temperature and humidity; air movement in

the room; radiation from the walls and surrounding objects; metabolic rate of the subject; time of day of the experiment; the experimental procedure; water intake, including volume, salt content, and temperature; diet; and the subject's physical condition and acclimatization to heat. Without the precise control of all these factors accurate comparisons of clothing are impossible. In field tests, a number of these factors cannot be controlled from one experiment to another and the observer must therefore rely to a large extent on the subjects' estimates of the clothing in regard to coolness, skin comfort, appearance, design, and so forth. The rates of sweating of subjects in standardized marches are the best of the above physiological criteria for use in the field. Protection from insects and trauma can best be determined in the field. The final evaluation of clothing should be based on both extensive field tests and laboratory tests. Field trials should be run if possible under the actual conditions for which the clothing is designed.

` FABRICS

In a field study carried out in the swamps of Florida in late summer, it was found that clothing made of thin (0.013-inch), tightly woven fabrics with low air permeability, such as Byrd cloth and Army poplin, served as effective barriers against mosquitoes. Army herringbone twill, 8.2-ounce twill, and the highly air-permeable British cellular weave, although nearly twice as thick as Byrd cloth, gave very little protection. All these are cotton fabrics. Protection from mosquitoes and other insects by means of clothing is important not only as an aid in prevention of malaria, dengue, and other diseases but also in protecting men from the actual bites. Soldier subjects preferred to wear suits made of Byrd cloth and poplin because they found them more comfortable and cooler than suits of other materials. In repeated walks over a standard course there was no difference in rates of sweating, rectal temperatures, and heart rates when they wore the different suits.

In subsequent laboratory studies, it was found that poplin jungle uniforms impeded the bodily movements of cross-country runners less than did herringbone twill uniforms. Eight trained runners were able to complete a standard 5¼-mile cross-country course in less time in poplin uniforms than in herringbone twill uniforms of the same design. The runners stated that they preferred running in the poplin suits because they offered less drag to the movement of their legs. This difference is undoubtedly due to the fact that poplin is a lighter, thinner, and smoother fabric than herringbone twill. The difference in smoothness and weight of the fabrics is thus a factor in fatigue. In addition, the smoother fabrics are probably less irritating to the skin.

Following the Florida field study a laboratory study was begun in an effort to determine if possible whether the soldiers were right in thinking

that Byrd cloth and poplin were cooler for tropical wear than the more permeable but thicker fabrics. Laboratory experiments on the above clothing worn by men walking on a treadmill with metabolic rates of 190 cal./ m.²/hr. were carried out under simulated jungle conditions; that is, a constant air temperature of 30.5° C. with 80 per cent relative humidity and a low air movement. With use of the physiological criteria of heat strain described above, the coolness of clothing was found to be related directly to the thinness of the cloth and inversely to the weight of the suit, the amount of water required to wet it, and the length of time required to dry it under constant conditions. In these fabrics, with air permeabilities ranging from 4 to 40 cubic feet per minute at a pressure equal to 0.5 inch of water, there was no advantage in increasing air permeability. The more permeable fabrics were even hotter than Byrd cloth and poplin because they were about twice as thick. These surprising results have since been confirmed, both in this country and in England.

An interesting discovery was as follows. A seersucker fabric having only three fourths the weight per square yard of the thin smooth fabrics, Byrd cloth and poplin, was found to be hotter than those fabrics. This difference was ascribed to the puckered surface of seersucker, which traps air into small pockets and gives the fabric an effective insulating thickness and resistance to vapor transfer greater than that of a smooth fabric of equal weight. Nylon fabrics had no special properties of coolness not possessed by cotton fabrics of equal thickness and weight. Additional experiments were run on the above suits under severe conditions of humid heat and under simulated desert conditions, with similar results.

It should be kept in mind that the above results apply to fabrics that are rugged and practicable for combat clothing. Even thinner fabrics several times more permeable are cooler and may be used to advantage in sedentary civilian occupations. Laboratory experiments revealed that impregnations of clothing with dimethyl phthalate for mosquito repellency or by the Chemical Warfare Service for protection from poison gases did not significantly increase the resistance of the clothing to the dissipation of body heat when worn by men working in humid heat.

Since evaporation is the major avenue of heat loss by men working in hot environments, it was essential that the above physiological results be correlated with physical measurements of vapor resistance of the fabrics. Workers engaged in this field of investigation developed a method of measuring the resistance of the fabric itself, separating this from the resistance of air spaces, since the rate of evaporation is determined by the heat supply and by the rate of diffusion of water vapor. With this method resistance of cotton and wool fabrics to water vapor transfer varied in only a small range with changes in density of weaving, being between two and four times the resistance of an equal thickness of air. In cotton and wool fabrics, the resist-

ance is much more influenced by the thickness of the cloth than by its porosity or openness of weave. These important observations explain the physical basis for the physiological results outlined above. Further experiments showed that with nylon, cellulose acetate, vinyon, and glass, all low in capacity to absorb water, the resistance to vapor transfer increases rapidly with increasing density of weaving, to values twenty or more times that of air. This finding and experiments on cellophane sheets indicate that water vapor passes through the substance of cotton and wool in significant amounts, as well as through the air spaces between fibers.

DESIGN, VENTILATION, EXTRA LAYERS OF FABRIC

In the first study of design of hot-weather clothing during the war, laboratory workers carried out a careful comparison of the coolness of one-piece and two-piece Army fatigue suits and found no significant difference between them when worn buttoned up. It was concluded, however, that the two-piece suit was preferable because it was more versatile; that is, when conditions warranted it the shirt could be removed, with a great increase in coolness and comfort. Under laboratory conditions men were significantly cooler with the skin exposed than when clothed, even with light fabrics. Similar observations on the advantage of exposing the skin of men exposed to indoor heat were made. Exposing the skin of the trunk and arms was seen to be more effective in keeping working men cool than exposing the legs by wearing shorts. Because of the danger of sunburn, exposure of the skin in outdoor heat must be practiced with caution, but here also when men were working hard they were cooler in shorts than when fully clothed.[1]

The same workers found that effective ventilation in hot-climate clothing was a distinct advantage in keeping men cool. Opening the collar and cuffs and turning up the trouser legs added appreciably to the ventilation through clothing. Unbuttoning the shirt was even more effective. Wearing a pack with a tight belt and shoulder straps greatly decreased ventilation. Special vents and net areas when limited to the axillas provided no appreciable ventilation.

A striking improvement in the coolness of hot-climate clothing can be effected by removing so far as practicable all extra layers of fabric. The Army jungle uniform and the summer flying suit of the Army Air Forces were found to be much cooler when extra pockets and gas-protective flaps were removed. Sleeveless undershirts and shorts added appreciably to the heat load of men working in humid heat. Brynje or net vests have been considered for wear underneath the summer flying suit by aviators flying from hot

---

[1] See below, page 512.

ground positions to cold atmospheres at high altitudes. Both field and laboratory experiments have proved that Brynje vests add significantly to the heat load of men working in hot environments but do not significantly raise the skin temperatures and rates of sweating of men resting in the heat. It is obvious that the chimney effect supposed to be effective in ventilating the skin of men wearing the vests in cold environments would not exist in warm environments, where the air temperature is about the same as the skin temperature; in extreme desert heat, convection might be in the reverse direction.

EFFECT OF WIND AND CLOTHING

Following their original observations that men working in humid heat were no cooler in porous British cellular weave than in Byrd cloth or poplin suits, a research group made a quantitative study of the effects of air movement and air permeability of clothing on the evaporative cooling of men walking at a constant rate on the treadmill. In air temperatures of 28, 34, and 46° C. varying the air permeability of clothing from 12 to 40 cubic feet per minute made no consistent difference in the rates of sweating, skin temperatures, and heart rates, nor did it alter to any significant degree heat exchange by the avenues of radiation, convection, and evaporation. A change in air movement from 5 to 184 m./min. made practically no difference in this relationship. At 28° C. the working men were more comfortable in shorts than in cellular weave or tightly woven suits, although there was little stress due to wearing the clothing; they sweated less, and the wind was more effective in reducing evaporative requirements. In each of the environments of 34 and 46° C. the heat stress as indicated by the above functions was less in shorts than it was in regular clothing. The stress imposed by wearing the clothing was reduced by lowering the air temperature, and at each air temperature it was reduced by increasing the air movement. Increasing the air movement, and thus convection, increased the heat stress on the men wearing shorts at 46° C., but even at the highest air movement the stress was less in shorts than in clothes. These results confirmed the original observations that the porous British cellular weave does not make cooler clothing than the thinner but more tightly woven fabrics.

Correlated with the above physiological data are the observations made on the effects of wind and fabrics on evaporative cooling. An artificial sweating apparatus, which could be dressed in different fabrics, was constructed and mounted in a wind tunnel. The material tested ranged from mosquito netting to the most tightly woven fabrics and cellophane and included experiments on the bare, wet artificial skin, which was made of saturated blotting paper. In still air the porosity of the fabric is of very little importance, since the resistance to diffusion is largely in layers of still air, which con-

tribute up to ten times as much resistance as the cloth itself. In moving air the resistance of the clothing and associated air layers falls off with increase in the velocity (V, miles per hour) or air permeability of the fabric (A, in cubic feet of air pushed through 1 square foot of cloth per minute by a pressure equal to 0.5 inch of water), so that the rate of evaporative cooling (E, in kg. cal. m²./hr./mm. vapor-pressure difference between skin and air, P) is

$$\frac{E}{P} = 3 + (0.3 + 0.004 \text{ A}) \text{ V.}$$

Any kind of fabric, even mosquito netting, cuts down the air movement and evaporation by a large factor. The evaporation from bare wet surfaces, such as the face and hands, is much larger than that through any fabric and is much more sensitive to air movement. Evaporation from wet fabric is similarly large and sensitive, but is less efficient in cooling the body than evaporation from the skin. This is correlated with the above data, which show that in hot environments the wearing of clothing greatly increases the physiological strain over that experienced when the skin is bare. Among the strong fabrics used for Army clothing, variations in air permeability from A = 50 down to zero make little difference unless there is much air movement. Hence, both the physical and physiological data show that tight weaving to prevent mosquito bites imposes no more heat burden than that presented by other fabrics such as herringbone twill, and if the fabric is thin may impose less burden.

Clothing in Relation to Radiation

Men exposed to the sun absorb radiation from the direct solar rays, from reflected sunlight, and from secondary radiation by ground and buildings. It has been found that the radiation absorbed in this way may be as high as 240 cal./hr., which is about three times the resting metabolic heat production. Clothing effectively shields a man from a considerable part of this heat gain. Herringbone twill fatigue suits saved resting men an average of 260 gm. of evaporation per hour when they were exposed to the desert sun and ground radiation. This represents a protection from 150 cal. of heat, practically all of it being from radiation. Laboratory experiments revealed the fact that khaki twill shirts, trousers, and sun helmets when worn by men sitting in the sun effected an economy of 165 to 202 gm. of sweat per hour over wearing of shorts only. In walking at a moderate rate on the treadmill in the sun (oxygen consumption about 1.3 l./min.), the men sweated at about the same rate when they were fully clothed as when they wore shorts. In performing hard work on the treadmill in the sun (oxygen consumption about 2.5 l./min.), they sweated 215 to 551 gm./hr. more when wearing the

twill suits than when wearing shorts. At the hardest grade of work the men stored considerable amounts of moisture in their clothing and evaporated 140 gm./hr. more in clothing than in shorts. The Indiana study showed that the protection from radiation provided by the clothing was an advantage to the men when they were at rest but a handicap in hard work, in spite of the protection it gave.

This relationship is probably dependent on several factors. In the first place, the clothing acted as a barrier to heat transfer in either direction; that is, to radiation toward the body and to the heat of metabolism from the body to the environment. At rest the protection from radiation by the clothing was greater than the metabolic heat, especially since the observations were made near midday and more radiation impinged on the subjects in the sitting position than in the erect walking position. A third factor is that in the hardest work the metabolic heat was relatively much greater than the protection from radiation afforded by the clothing. Well-ventilated tropical helmets saved the men an average of 55 gm. of sweat per hour when they were sitting or walking in the sun. In addition to this economy of sweat, the helmets shaded the eyes and kept the tops of the men's heads about 6° C. cooler than when they were bareheaded. From these results it was concluded that when exposed to solar radiation in a hot climate it is advantageous to wear a ventilated helmet during both work and rest; it is economical to wear clothing in light work and at rest; in hard work there is economy of heat exchange in wearing only shorts, shoes, and a sun helmet, provided the skin does not sunburn.

Later experiments showed that khaki twill jungle uniforms were cooler on men marching in the summer sun than O.D. jungle uniforms made of the same fabric. This difference is due to a difference in the absorption of radiation by the two dyes, as shown by the observation that khaki twill reflects 56 per cent of the sun's radiation and O.D. herringbone twill only 26 per cent. In exposure of men to indoor artificial radiation from a bank of mazda lamps no physiological difference between the two colors of fabrics was detected.

FOOTWEAR

The care of the combat soldier's feet is one of the major problems of tropical warfare. A completely satisfactory combat boot for jungle warfare has not been developed. An all-leather shoe appears to be the best type available in the opinion of many experienced soldiers. There are disadvantages to rubber, even in the sole of the shoe; it is impermeable, and according to one investigator it never forms itself to the weight-bearing points of the foot and therefore is never comfortable for standing or marching. High-topped boots are a disadvantage in that they cause irritation and overheating of the leg, and this may contribute to the development of jungle sores so prevalent

around the ankle and lower leg. The best sock for marching in hot climates is one with a fairly heavy woolen knit sole and a light cotton knit upper. The cushion-sole sock of the Office of the Quartermaster General is an excellent development along this line. Wearing heavy woolen socks around ankles and legs leads to local prickly heat. Experiments by the Army Air Forces at Eglin Field, Florida, showed conclusively that ventilation of the feet obtained by wearing sandals prevents as well as cures fungus infections of the feet, which are so prevalent in hot climates. This is a practical procedure for all personnel in the tropics except the soldier in actual combat, and even he might make some use of it.

Laboratory studies made it evident that in men walking on the treadmill friction between the layers of the shoe sole and between the shoe and the plantar surface of the foot can cause considerable elevation of the skin temperature. The temperature of the soles increased as the speed of walking increased; with the speed constant, the temperature increased as the weight of the load the subject carried was increased. It is known that friction was the principal factor in raising the temperature in these experiments, since in some experiments in rapid walking the skin temperature of the foot rose above 43° C., a level well above that of the blood temperature and the temperature of the treadmill surface. These facts revealed that serious consideration must be given to speed of marching in relation to the load carried in order to avoid blisters and other casualties involving the feet.

Comparative experiments were conducted in which the subjects wore tennis shoes, Army jungle boots, Army service shoes, and Army combat boots alternately while walking on a treadmill in a hot environment. The weights of these shoes were respectively 700, 1300, 1650, and 2200 gm. The addition of 1 kg. to the weight of shoes increased metabolism as much as the addition of 4 kg. of weight to the pack with the subject walking at 3.5 m.p.h. The strain on men walking in the heat, as measured by their heart rates, rectal temperatures, skin temperatures, and rates of sweating, varied directly with the weight of the shoes. In critical experiments in which the men walked in the heat without drinking water they could walk significantly longer and remain in better condition in the light shoes than in the service shoes. It was concluded that for hot climates the weight of materials used in the construction of footwear should be limited to that absolutely necessary to fulfill the requirements of protection and durability.

WATER-RESISTANT CLOTHING

Water-resistant fabrics are needed for wet-weather and wading garments and for exposure suits; the Chemical Warfare Service requires impermeable suits in decontamination work. These fabrics may vary in the degree of water repellency from shedding rain during short periods to complete im-

permeability, including not only resistance to high water pressures but even prevention of the passage of water vapor. In addition to the protection from water provided by these materials, their use requires a consideration of the effect on evaporation of perspiration. For men working in warm or hot environments where evaporation is the principal avenue of heat dissipation, prevention or impairment of evaporative cooling is a limiting factor in their tolerance.

WATER-REPELLENT CLOTHING

During the war work at a university laboratory was first directed toward making water-repellent fabrics more resistant by increasing the contact angles of water against the repellent fibers and by decreasing the pore size. The highest contact angle achieved was 140°, and the smallest pore size that the textile industry could produce was 8 to 12 μ in "Jo" cloth. This combination gave a resistance of 130 cm. of water pressure, which is not sufficient to provide complete protection from most types of exposure to water; even this degree of resistance could not be maintained for long periods of exposure. With the best repellents developed the only way to get higher water resistance than this was to produce fabrics with smaller pore sizes, and the investigators concluded that there was little immediate prospect of the textile industry's weaving fabrics with pores sufficiently small to provide a suitable water resistance to keep men dry when immersed in water.

Experimental investigation has revealed that water repellency does not significantly increase the resistance of clothing to heat dissipation even in extremely hot, dry environments where evaporation is the only means of dissipation. In laboratory experiments, working men wearing water-repellent poplin clothing in both hot humid and hot dry environments for periods of time up to five hours were under about the same physiological strain as when they wore water-absorbent poplin clothing under the same conditions. The average rates of sweating, percentage of sweat evaporated, skin temperatures, rectal temperatures, and heart rates were practically the same in every comparison of the two garments. Results of the same investigators in field tests conducted on men marching in the summer sun at Fort Benning, Georgia, agreed closely with these findings. The average rates of sweating of twelve men were practically the same in the two types of uniforms. Nine of the twelve men who compared the uniforms in the field test preferred water-repellent poplin because it made them cooler. Water-repellent clothing did not absorb as much sweat as the absorbent garments and thus could be dried faster. Correlated with these physiological results is the observation that water-repellent treatments on the surface of cotton fibers do not impair their permeability to water vapor. Because of these results, we can conclude that water-repellent clothing can be worn continuously with safety by combat

soldiers in any climate where it may be useful as protection from rains or where its rapid drying quality is helpful. In civilian life such fabrics will serve well in shower-proof garments where exposures are short.

IMPERMEABLE CLOTHING

The Chemical Warfare Service finds it necessary to use decontamination suits that are impermeable to water vapor, and these suits definitely limit evaporative cooling. Laboratory experiments brought out that men were able to perform the average work of decontamination for only one hour in an atmospheric temperature of 24° C. and for only twenty-five minutes when the air temperature was 29.5° C. Keeping the outer surface of the impermeable suit wet more than doubled the tolerance time. It was found that impermeable outer garments could be worn by men performing hard work in cold environments for two-hour periods without immediate adverse physiological effects, but that a higher percentage of the perspiration was not evaporated and remained in the clothing than in similar experiments where the outer garment was permeable. This shows that more frequent drying of the clothing is necessary if impermeable outer garments are worn for long periods in cold environments.

A comparison was made of raincoats and ponchos in order to determine the effects of material, design, and ventilation of these garments on the heat loss of men working in a simulated jungle atmosphere. Under these conditions all rain garments made the men sweat more. Ponchos were found to be much cooler than raincoats as judged by the rates of sweating and skin temperatures. A special "arcovent" design of raincoat, in which large areas in the regions of the shoulders and axillas are covered only by a loose yoke, was of doubtful value in keeping the men cool. Water vapor permeability provided by water-repellent fabrics was a much more important factor in coolness of raincoats than thinness and light weight of the fabric or than ventilation provided by special arcovent design. It was recommended that for prolonged exposures of troops to rain an impermeable poncho should be used in preference to a raincoat. The poncho is versatile in its uses; it can be worn over the soldier's pack or employed as a shelter half, whereas the raincoat cannot. In warm weather a rain garment to be used by personnel who require protection during short exposures only should be of water-repellent rather than impermeable material.

EXPOSURE SUITS

An exposure suit is a water-resistant coverall designed to prevent wetting of the underlying clothing when a man is immersed in water or is waiting for rescue in an open boat exposed to spray. It was established as the result

of laboratory experiments that human beings, whether naked or clothed, react according to a pattern when they are in water of various temperatures. Briefly, when the water temperature is above 15° C. the subject can increase his heat production sufficiently by shivering to establish an internal temperature only 1 or 2° C. below the normal. The colder the water in the range above 15° C. the more he will shiver, and accordingly the sooner the great physical effort exerted will cause exhaustion and rapid loss of body heat. In water colder than 15° C. progressive cooling occurs in spite of the most vigorous shivering. Again, the colder the water the more rapid is the loss of body heat, and in water near the freezing point death occurs in a few minutes.

The ordinary clothing textiles, for our purposes, provide a means of enmeshing air in such small particles that a layer of nearly still air is formed. This layer acts as a thermal insulator, the effectiveness of which is proportional to its thickness. A man clad in the Army Air Forces heavy winter clothing assembly is sufficiently insulated to withstand a temperature of − 1° C. for many hours. Since no ocean water is colder than this, it should be possible to protect a person immersed in the coldest water by mere avoidance of displacement by water of the air contained in the clothing. Both laboratory and field trials by various workers demonstrated that protection against very cold water could be obtained by having a man wear an impermeable suit outside of the insulating clothing. Exposure suits impermeable to water were then designed by the manufacturers to meet the many technical details involved in devising a garment that was watertight and at the same time could be put on very rapidly and did not interfere with agility. Designs that were satisfactory for both bomber crews and aviators flying one-man planes were worked out and accepted by both the Army and the Navy. In laboratory experiments it was found that in cold environments an impermeable exposure suit worn over the clothing is an advantage to a resting or slightly active man in exposures of several hours, especially if his inner clothing is wet to begin with. Since the amount of underlying clothing needs to be selected on the basis of the temperature of water in which the aviator may be immersed and the atmosphere to which he may be exposed in flight, and since a pursuit pilot must be completely attired before he leaves the ground, there are many situations that cause aviators to be far too heavily dressed while waiting for flights. In warm or hot environments, where most of the body heat must be dissipated by evaporation, the addition of an impermeable exposure suit over the other clothing may add appreciably to the heat stress. This difficulty could be overcome by spraying the outside of the impermeable suit with water, the evaporation of which caused adequate cooling while the aviator was waiting to take off. Later, after investigators had discovered that they could not make woven fabrics water-resistant enough for exposure suits, they began studying the newly developed lami-

nated "Aerobond," and from this they developed a textile that prevents the passage of water but permits the transfer of water vapor. Exposure suits constructed of this textile were shown to afford the desired protection against wetting and at the same time to permit the evaporation of moderate amounts of body moisture.

In this fabric pore sizes as small as desired could be secured by binding together into a fabric finely divided particles of hydrophobic silica aerogel. The particles could be held together between two layers of a thin fabric by natural latex or by a synthetic latex of the GRS type. This gives a thin, flexible fabric with sufficient vapor permeability to allow men to be comfortable when resting in fairly hot environments. In Aerobond the water resistance increases as the pore size in the aerogel layer is made smaller by use of smaller particles of silica gel. The vapor permeability becomes lower as the pore size is decreased and the rubber content is increased or as either occurs alone, but even with water resistance high enough to provide protection of men immersed in water the vapor permeability is still great enough so that resting men can wear the suits continuously, even in warm environments, and have adequate evaporative cooling.

Aerobond fabrics may also be used in rain-protective garments and waders. The density of the finely divided hydrophobic silica aerogel is low — 3 to 9 pounds per cubic foot — and this is one of the best thermal insulators known. Hence, by varying the thickness Aerobond fabrics may be used to provide warmth or buoyancy or both, as well as water resistance and vapor permeability. This extraordinary fabric represents one of the most important and interesting developments in the field of protective clothing during the war.

PRACTICAL APPLICATIONS

A number of the investigations under the sponsorship of the Committee on Medical Research not only improved the comfort and efficiency of men exposed to various environmental stresses but in some cases helped to prevent injury, disease, and loss of life. It must be kept in mind that in addition to physiological considerations there are many other problems involved in equipping an army. Technical difficulties of production and supply of materials are examples of other factors that determine whether or not particularly advantageous articles may be provided. The following are a few examples of the scientists' contributions.

Scientific information provided by physiologists and textile experts in correlation with field practice led the Army to adopt many new improvements in cold-weather equipment such as sleeping bags, clothing, and footgear. The development and use of quickly donned and continuous-wear exposure suits represent another instance where scientists played a prominent part.

Improved electrically heated suits were adopted by the armed forces as a result of studies of the scientists. Thin, tightly woven mosquito-protective fabrics were used for hot-climate clothing and for summer flying suits after extensive physiological and physical laboratory studies on a number of different fabrics had been carried out. Several field trials in the United States and Panama by the armed forces and finally a trial by two Army divisions in combat in the Pacific Theater emphasized the advantage of these fabrics over others. As in many other instances, difficulties in production of material delayed the adoption of tightly woven fabrics as hot-climate clothing.

In addition to the developments of clothing, this research has provided a large store of fundamental knowledge related to clothing and climatic problems.

The studies of clothing made under the sponsorship of the Committee on Medical Research were greatly facilitated by the guidance of the Subcommittee on Clothing of the National Research Council. The Research and Development Branch of the Office of the Quartermaster General presented numerous problems, provided experimental clothing and equipment for any investigations the laboratories wished to make, organized field studies for practical tests of clothing, and arranged to have the important developments tried by large groups under combat conditions. The Navy and the laboratories of the Army Air Forces functioned in the same way with their clothing problems. Since this chapter has dealt only with the activities of the Committee on Medical Research, we wish to emphasize that extensive investigations of clothing problems were also carried out by a number of service laboratories. The investigators are especially indebted to the soldiers and Civilian Public Service men who acted as subjects and technical assistants in the studies.

CHAPTER XXXIV

WATER DISINFECTION AND ALLIED SUBJECTS

GORDON M. FAIR

THE PROJECTS covered in this chapter are as follows: water disinfection and allied subjects (Harvard University); sterilization of water in canteens (University of Southern California); and fundamental biochemistry of water disinfection (Columbia University).

Although the technics for disinfection of large-scale water supplies are well standardized, they are not applicable to the treatment of water under field conditions of global warfare. The problem of assuring rapid and reliable protection to troops on their own resources, who may be forced to draw their supplies of drinking water from sources exposed to serious contamination or pollution with any one or all of the wide variety of water-borne pathogenic agents, is far more difficult than that encountered in ordinary municipal practice. Disinfection cannot be preceded by coagulation and filtration and must be effective without substantial modification of directions in all types of waters likely to be encountered.

Historically, calcium hypochlorite ($Ca(OCl)_2$) in cans or ampoules has been employed for the treatment of water supplies in the field in units down to the size of the Lyster bag (34 gallons) and the 5-gallon can. Tablets containing halazone (*p*-dichlorsulfonamidobenzoic acid) have generally been used for the treatment of water in canteens, and tincture of iodine has occasionally served for this purpose. As employed in the past, all these substances may furnish only partial protection against infection. The studies herein reported were therefore undertaken with the objective of developing more adequate agents or methods of emergency water disinfection. Attention was directed not only to a practical examination of disinfecting agents already commercially available, but also to a basic study of the principles and mechanism of disinfection, in order that more powerful agents might be developed.

Of the known water-borne pathogens — typhoid, dysentery, and cholera bacteria; cercariae of flukes causing schistosomiasis; cysts of *Endamoeba histolytica,* producing amebic dysentery; leptospirae, associated with infectious jaundice; and the virus responsible for infectious hepatitis — the cysts of *E. histolytica* appear to be the most resistant to almost all the known disinfecting agents. The resistance of infectious hepatitis virus, however, has not as

yet been tested over a sufficiently broad range of conditions to determine its exact position in the scale of disinfection. On the basis of available information, it was assumed that the limiting factor in the use of most disinfecting agents was their ability to destroy amebic cysts. Hence, the studies herein reported center about this organism.

The disinfecting agents studied comprised chlorine and chlorine compounds releasing hypochlorous acid in solution, bromine and its compounds, iodine and systems releasing iodine, iodine chloride, chlorine dioxide, peroxides, organic mercurials, salts of silver, and synthetic detergents. Of these only chlorine and its compounds, iodine and its compounds, and the synthetic detergents proved promising enough for detailed study. Of the other substances, bromine and its compounds were eliminated from intensive investigation because of their rapid inactivation in the presence of organic matter, and the remainder because they were not sufficiently cysticidal.

CHLORINE AND CHLORINE COMPOUNDS

Dilute solutions of chlorine in water hydrolyze completely in less than a second, according to the equation

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-.$$
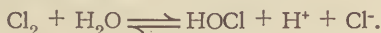
In practice, therefore, water disinfection is never accomplished by chlorine itself, but by its hydrolytic products. Hypochlorous acid is also produced when any of the hypochlorites (calcium hypochlorite, sodium hypochlorite, bleaching powder, and so forth) are dissolved, as shown by the typical equation

$$Ca(OCl)_2 + 2H_2O \rightleftharpoons 2HOCl + Ca^{++} + 2OH^-.$$
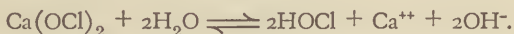
Halazone and many of the other N-chloro compounds also yield hypochlorous acid by hydrolysis, although in this instance only a part of the chlorine present may react. With halazone the reaction is

$$HOOCC_6H_5SO_2NCl_2 + H_2O \rightleftharpoons HOOCC_6H_5SO_2NHCl + HOCl.$$

The hypochlorous acid formed in all three cases is a weak acid and ionizes according to the equation

$$HOCl \rightleftharpoons H^+ + OCl^-,$$
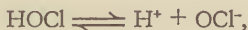
the extent of the reaction depending on the pH of the solution. At pH 5 practically no ionization occurs; at pH 11 ionization is almost complete; the fraction ionized at intermediate pH values can be calculated from the ionization constant. It has long been assumed, and it was quantitatively demonstrated in the Harvard project, that the disinfecting action of solutions of chlorine and its compounds is to be attributed almost entirely to the presence of hypochlorous acid, the effect of either OCl^- ion or of unhydrolyzed

N-chloro compound being comparatively very small. Since the standard methods for the estimation of chlorine in water give equal weight to hypochlorous acid, OCl⁻ ion, and chlorine bound to nitrogen, figures for titrable or available chlorine mean little as regards disinfecting power unless the pH and other conditions are known.

The enormous effect of hydrogen-ion concentration on the relative quantity of hypochlorous acid present in chlorine solutions is shown in Table I. The figures indicate that it should require about thirty times as

TABLE I

Effect of Hydrogen-Ion Concentration on Relative Amounts of Hypochlorous Acid Present in Dilute Chlorine Solutions at 20° C.

pH	5	6	7	8	9	10
Titrable chlorine required to produce unit HOCl	1.00	1.03	1.33	4.3	34	331

much titrable chlorine to destroy cysts at pH 9 as at pH 5. Experimentally determined titrable chlorine residuals for the destruction of cysts at a concentration of 30 per milliliter follow this pattern very closely, as shown in Figure 65. The solid curves in this diagram were drawn from theoretical equations, assigning to OCl⁻ ion a disinfecting efficiency of 0.0025 of that of hypochlorous acid at 23° C. and 0.0061 at 3° C.

Even in the acid range, the titrable chlorine residual required to destroy thirty cysts per milliliter ranged from 2.8 ppm at 23° C. to 20 ppm at 3° C. for a ten-minute contact period, and from 1.3 ppm at 23° C. to 7.5 ppm at 3° C. for a thirty-minute contact period. These values are substantially higher than the residuals that have commonly been employed in the past for water disinfection, except where superchlorination or breakpoint chlorination [1] has been used.

The bactericidal efficiency of dilute chlorine or hypochlorite solutions, too, is reduced by dissociation of hypochlorous acid at high pH values. Experimentally, however, this effect could not be evaluated with the same quantitative accuracy as for cysts, because the vegetative bacteria are extremely sensitive to chlorine, and dosage must be held to very small concentrations of hypochlorous acid. At pH 7, for example, 1,000,000 *Escherichia coli* per milliliter of otherwise clean water are reduced to less than 5/100 ml. in ten minutes by 1 ppm of residual titrable chlorine. The destruction of typhoid, paratyphoid, Shiga dysentery, and cholera organisms was shown to be at

---

[1] Breakpoint chlorination is controlled superchlorination in which sufficient chlorine is added to destroy all the ammonia present and to leave a slight residual quantity of hypochlorous acid.
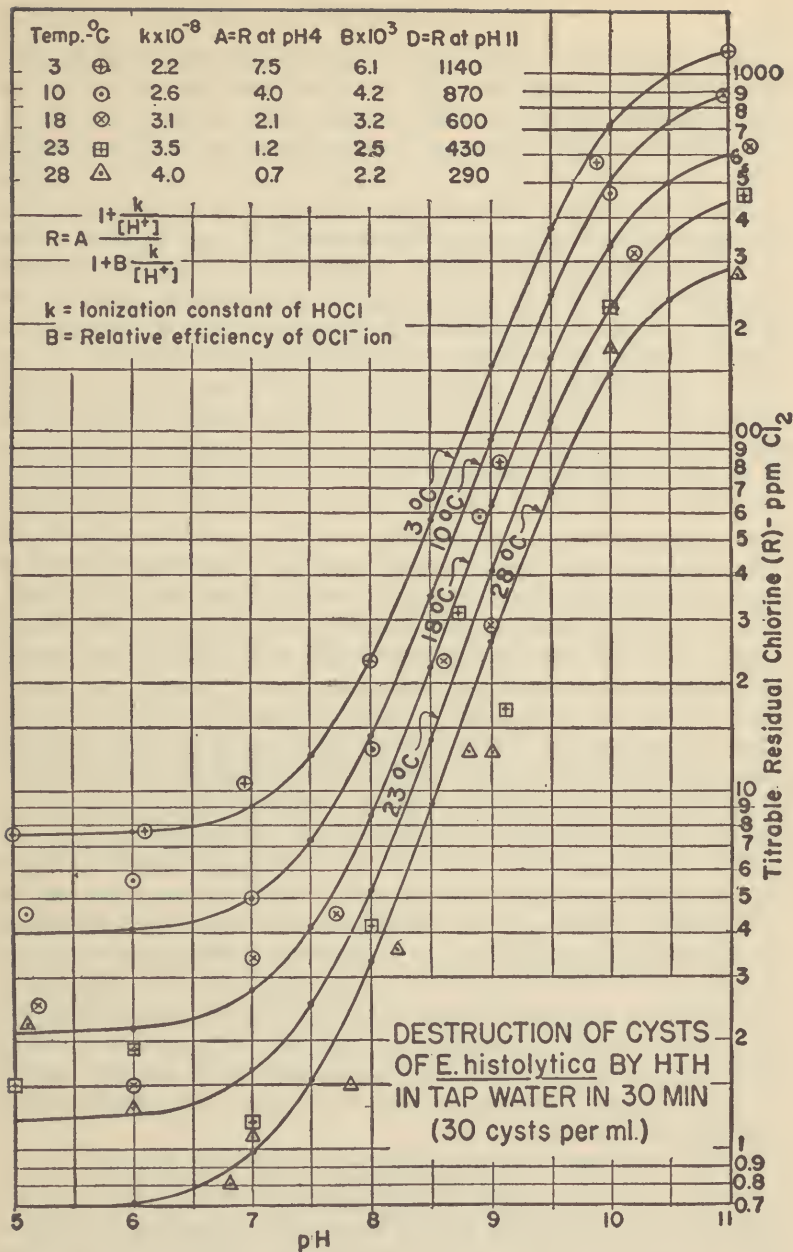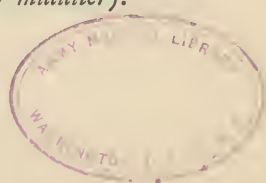
FIGURE 65. *Destruction of cysts of* Endamoeba histolytica *by HTH in tap water in 30 minutes (30 cysts per milliliter).*

least as great. In water containing particulate matter that cannot be pene-
trated by the chlorine the reduction was much less efficient, but this is a
serious limitation that inheres in all chemical disinfectants.

The cercariae of the schistosomes are reported to be killed by a residual
titrable chlorine dose of 1 ppm in about thirty minutes, and this is also a
reported requirement for inactivation of the virus of infectious hepatitis
after removal of particulate matter. Experiments at Harvard have shown that
destruction of a purified preparation of Theiler's mouse poliomyelitis virus
is accomplished by chlorine residuals in the neighborhood of 1 ppm. It was
also found that the leptospirae causing infectious jaundice are even less re-
sistant to hypochlorous acid than are the pathogenic enteric bacteria.

These required chlorine residuals constitute sufficient dosages of the dis-
infectant only if the water to be disinfected contains no foreign organic
matter, or reducing substances, in addition to the pathogens. Two important
side reactions of hypochlorous acid, which increase the required disinfecting
dose, occur in most natural waters. These are reactions with oxidizable
organic matter to reduce the hypochlorous acid to chloride, and reactions
with ammonia or nitrogenous material to produce chloramines. The first of
these — the chlorine demand — must be satisfied before any reliable disin-
fecting residual can be obtained. Experiments have shown that this demand
is closely associated with the tannins and other vegetable coloring materials
that stain water; that with highly colored waters the demand in ten minutes
may be as high as 10 to 15 ppm of titrable chlorine; and that the demand is
higher in more basic solutions. Formation of chloramines, while leaving the
concentration of titrable chlorine unchanged, nevertheless reduces its disin-
fecting power because the chloramines are weaker disinfectants. Investiga-
tions by the United States Public Health Service have indicated that the
titrable residuals for chloramines must be twenty to forty times as great as
those for hypochlorous acid to achieve complete kills of vegetative bacteria
in equal contact periods. For cysts, the difference has been shown at Harvard
to be considerably less, dichloramine being 60 per cent as effective as hypo-
chlorous acid and monochloramine 22 per cent as effective. Because of OCl^-
ion formation at high pH values, the chloramines are actually more effi-
cient cysticides at pH values above 7.5. This is a very significant finding,
which leads to the conclusion that the formation of chloramines may be
desirable at high pH values unless the hydrogen-ion concentration itself
can be brought under control.

For field disinfection, where organic matter cannot be removed prior to
treatment and where reliable laboratory control cannot be secured, doses
must be fixed at sufficiently high values to allow for maximum chlorine
demand and chloramine formation. At these values, unfortunately, the water
becomes unpalatable unless the excess chlorine is removed by dechlorinating
agents after disinfection has been achieved.

The analysis of existing information and the new information obtained in the course of these studies led to the conclusion that calcium hypochlorite or other hypochlorites could give completely satisfactory results in field disinfection only if suitable modifications to existing practices were adopted, as follows:

(1) A dose of 25 to 30 ppm of titrable chlorine should be employed to ensure a residual of 7.5 ppm of titrable chlorine as hypochlorous acid after satisfaction of the chlorine demand and formation of chloramines.

(2) Since disinfection will not be assured even with these doses unless the pH is below 7.5, and since addition of the required amount of commercial calcium hypochlorite causes a pronounced increase in the pH of the water, acidification of the water to be chlorinated is necessary. This can best be accomplished by adding solid acids along with hypochlorite. A dose of 50 ppm of citric or tartaric acid or 100 ppm of acid phosphates or sulfates was found to ensure a pH below 7.5 in most waters that might be encountered in the field.

(3) Dechlorination is necessary after disinfection has taken place to make the water palatable. This should be done as the water is drawn into the individual canteens, so that the water held in the Lyster bag will be protected against recontamination. Tablets of sodium sulfite could be used for this purpose, 1.8 mg. of sodium sulfite being equivalent to 1 mg. of chlorine.

Since hypochlorites are not readily tabletized, several N-chloro compounds were investigated as possible substitutes for canteen sterilization. Those studied, together with the chemically determined percentages of titrable chlorine liberated as hypochlorous acid in solution, were: halazone (50 per cent), N-chlorsuccinimide (0–10 per cent), dactin (40 per cent), chloramine-B (0–5 per cent), chloramine-T (0–5 per cent), hexachloromelamine (30 per cent), and N-chloracetamide (0–1 per cent). The cysticidal doses were always the amounts required to liberate a cysticidal amount of hypochlorous acid. This indicates at most a very slight effect of the N-chloro compounds themselves. Thus, with halazone twice as much titrable chlorine was required to kill cysts as with hypochlorite at the same pH, and with N-chlorsuccinimide fifteen to twenty times as much. Tablets of these materials were even less efficient because their dissolving times were appreciable. On the whole, therefore, chlorine compounds cannot be considered satisfactory for use in situations requiring treatment by the addition of a single tablet to water.

IODINE AND IODINE COMPOUNDS

Iodine is much less hydrolyzed than chlorine in water, and at the required dosages for field disinfection the formation of HOI is very slight at pH values below 7.5. Under practical conditions, therefore, disinfection is accomplished by the reaction of iodine with the pathogen. Since iodine does

not react with ammonia or nitrogenous substances to form iodoamines in dilute solution, the disinfecting efficiency is measured directly by the titrable iodine present.

The concentration of iodine required to destroy cysts was again found to be the limiting factor in setting the dosage for field disinfection. For a contact time of ten minutes it was found that 4 ppm of iodine was required to destroy 60 cysts per milliliter at 23° C., and 8 ppm were required at 3° C. The cysticidal dosage was independent of pH up to a value of 7.5.

A large number of bacteriologic tests indicated that in otherwise clean water a dose of 3 to 5 ppm of iodine would in ten minutes reduce 1,000,000 enteric bacteria per milliliter to less than 5/100 ml., including *Esch. coli, Shigella dysenteriae, Eberthella typhosa, Salmonella schottmülleri,* and *Vibrio comma*. Similar results were obtained with natural coli-aerogenes organisms in a mixture of tap water and 10 per cent sewage that had been strained to remove particulate matter. This efficiency was maintained at pH values up to 8 and at temperatures above 10° C. Below this temperature, twenty minutes was generally required to give satisfactory disinfection at these doses.

The cercariae of the schistosomes, as shown in tests at the National Institute of Health, are killed by smaller doses of iodine than are amebic cysts. The leptospirae of infectious jaundice were found to be killed by 0.5 ppm of iodine in five minutes. No results are available on the destruction of the virus of infectious hepatitis, although tests on epidemic poliomyelitis virus have shown approximately equal effectiveness for iodine and chlorine.

Although disinfection by iodine is not affected by ammonia or amino acids, iodine undergoes reaction with reducing organic substances, giving an "iodine demand" which for many waters was found to be of the same order of magnitude as the chlorine demand. A dose of 7.5 to 8 ppm of iodine was found to be sufficient to care for the demand of the great majority of waters, except highly colored swamp waters, and to leave sufficient residual for disinfecting action in ten minutes for all except very cold waters.

Iodine itself is quite insoluble in water, but compounds — triiodides or periodides — have been prepared that are readily soluble and release iodine in elemental form as soon as dissolved. In addition it has been shown that iodine may be made soluble by incorporating it in finely divided form in certain water-soluble cellulose derivatives. The use of tincture of iodine is not desirable because of inability to control dosage accurately with a liquid reagent and because of objectionable taste and odor.

A large number of triiodides were investigated or synthesized, and several were found to be stable and soluble. They were incorporated successfully in tablets containing disodium dihydrogen pyrophosphate as an acid-buffering agent and filler, in quantities sufficient to liberate 8 mg. of iodine per tablet

in solution. Such tablets appear to be the most satisfactory means so far developed for water disinfection in canteens.

The triiodide selected as having the most desirable combination of properties was a newly synthesized compound, triglycine hydroperiodide. Tablets containing this substance as the disinfecting agent have been shown to be stable under normal conditions of storage and use if properly packaged, to dissolve in less than a minute, and to disinfect the great majority of water supplies in ten minutes with the use of one tablet per canteen. Two tablets are required for highly colored waters, and a twenty-minute disinfecting period for very cold waters. High turbidity, alkalinity, ammonia, urea, and salt had no appreciable effect on the disinfecting efficiency. Field tests by various groups in the armed forces showed an overwhelming preference by the men for triiodide-containing tablets over halazone, chlor-dechlor, iodine-bromine mixtures, and tincture of iodine, chiefly on the bases of palatability, rapidity of disinfection, and convenience of application.

Possible toxicity of iodine at probable intake levels was thoroughly considered. A survey of the literature and feeding tests on rats by Otto Krayer of the Harvard Medical School indicated that difficulties would be encountered very rarely, if at all. This was also the expressed opinion of medical advisors to the Committee on Medical Research. Studies carried out at the Armored Medical Research Laboratory showed that six weeks of high-level intake of iodine-treated water by men undergoing physical exertion under simulated tropical conditions produced no noticeable chemical disturbances, metabolic changes, or interference with work performance.

EVALUATION OF DISINFECTANTS

The pronounced success achieved in calculating the cysticidal efficiencies of mixtures of hypochlorous acid and OCl^- ion and of $NHCl_2$ and NH_2Cl in terms of the efficiencies of the individual substances raised the possibility of assigning to each disinfecting species a number that would represent quantitatively its cysticidal efficiency, alone and in mixtures with other disinfectants. The number chosen to represent this "cysticidal constant" was the number of liters of water at 23° C. containing 30 cysts per milliliter that can be disinfected by 1 gm. of titrable halogen from the disinfectant. This particular form of constant allows the disinfecting efficiency of mixtures to be calculated by a simple additive relation.

A number of cysticidal constants are shown in Table II. If, for a mixture of hypochlorous acid and OCl^- ion or of NH_2Cl and $NHCl_2$, the sum of the products of the ppm of each species and the corresponding cysticidal constant is greater than 1000, the solution is cysticidal under the specified conditions. If the result is less than 1000, not all cysts will be destroyed. It is

hoped that this may prove to be a general relation, but it has not yet been adequately tested for other mixtures.

<div align="center">TABLE II</div>

<div align="center">Cysticidal Constants for Halogen Compounds</div>

Substance	10-minute Cysticidal Constant	30-minute Cysticidal Constant
HOCl	358	833
OCl —	1.1	2.1
NHCl$_2$	167	500
NH$_2$Cl	—	182
HOBr	<250	—
HOI	∼ 67	—
I$_2$	285	—
Chloramine-B	< 4	—
Chloramine-T	< 7	—
N-chloracetamide	< 5	—

SYNTHETIC DETERGENTS

Only those synthetic detergents in which the surface-active portion of the molecule carries a positive charge — the so-called cationic detergents — have been shown to be powerful general disinfectants. Since they are all completely substituted ammonium-type compounds, they are also referred to as the quaternary ammonium detergents. They may be crystalline or waxy solids and for the most part are soluble in water. The cationic detergents possessing disinfecting powers all have a higher hydrocarbon radical, generally containing twelve to eighteen carbon atoms as one of the substituents on the ammonium ion.

Comparative studies were made at the University of Southern California of the efficiency of fifty-seven detergents against *Esch. coli* and the cysts of *E. histolytica*. Relative disinfecting efficiencies were evaluated by means of ED$_{50}$, the dilution at which just half the culture tubes showed no growth. The toxicity of the substances was also investigated.

Practically all the compounds tested showed some cysticidal activity at room temperature and at cyst concentrations of 1000 per milliliter. Five of the compounds gave ED$_{50}$ values of 1:70,000 or better. In the order of their efficiency these were: (*a*) 1-*n*-tetradecyl-4-methylpyridinium chloride (Upjohn KIII–234); (*b*) *n*-hexadecyl-dimethyl-(β-hydroxyethyl)-ammonium chloride (Ortho 244); (*c*) *n*-hexadecyl-di-*n*-butyl-(β-hydroxyethyl)-ammonium chloride (Ortho 258); (*d*) 1-*n*-hexadecyl pyridinium chloride (Ceepryn); and (*e*) *n*-hexadecyl trimethyl ammonium bromide (Cetamium). Compound *a* was not considered promising, however, because of

rather high toxicity to mice; the other substances listed were all relatively nontoxic. Tests on compounds *b, c,* and *d,* as well as a number of others, were conducted at Harvard University by the same methods as were employed with the halogens. With 30 cysts per milliliter at 23° C. for ten minutes the cysticidal doses of Ortho 244 and Ortho 258 were 5 to 10 ppm; for Ceepryn, Fixanol (cetyl pyridinium bromide), and Sapamine KW they were 15 to 25 ppm; other materials tested required higher doses.

Nearly all the fifty-seven detergents studied at Southern California were also shown to have considerable activity against *Esch. coli,* tests being conducted with 20,000,000 bacteria per milliliter at 20° C. for ten minutes. However, the best cysticides were not the best bactericides, and none of the soluble compounds exhibited an ED_{50} better than 1:45,000. A great many approached this value. A special test, in which a pathogenic strain of *Salmonella enteriditis* treated with 1:10,000 Zephiran Chloride (alkyl dimethyl benzyl ammonium chloride) was fed to mice, showed that the action of this detergent was not reversed in the animal. In tests at Harvard 1,000,000 *Esch. coli* per milliliter were reduced to less than 5 per 100 ml. in ten minutes by 25 ppm of Fixanol and by 37.5 ppm of Ceepryn. This reduction, however, was not reached even with 100 ppm of Ortho 244.

On the basis of these tests the minimum dose required for water disinfection is 25 to 50 ppm of Ceepryn or Fixanol. Solutions of this concentration foam badly and have a flat, bitter taste. Hence the use of synthetic detergents cannot yet be recommended for water disinfection, although they are attractive materials in that their activity does not seem to depend on pH or the presence of organic matter.

THE MECHANISM OF DISINFECTING ACTION

The low concentrations at which the halogens act on unicellular organisms indicate that the disinfecting mechanism is not an oxidative destruction of the organism as a whole, but rather a specific reaction with some vital portion of the cell. Two processes are believed to be involved: the rate of diffusion of the disinfectant through the cellular membrane or wall, and the reaction with intracellular substance after penetration.

The studies at Harvard University led to the conclusion that the factor determining the difference in resistance of different types of organisms and the difference in efficiency of various halogen compounds may be correlated with the resistance of the cell wall to diffusion. From this point of view the greater resistance of cysts to disinfection as compared with bacteria is accounted for by the thickness and resistance to diffusion of the cystic membrane. The differences in efficiency of hypochlorous acid and OCl^- ion and of I_2 and I_3^- ion with respect to cysts can be attributed to the relative ease of diffusion through the cyst wall of neutral molecules as compared with nega-

tive ions. The greater efficiency of hypochlorous acid as compared with *N*-chloro compounds such as succinchlorimide may be the result of the inability of the larger molecule to diffuse through the cyst membrane. From this point of view the search for better disinfectants should focus on small neutral molecules possessing the requisite reactivity.

Reactions inside the cell wall were investigated at Columbia University. Studies of the relation between the destruction of bacterial enzyme systems and the ability of bacteria to reproduce demonstrated that the bacteria were killed at just the concentration of halogen disinfectant required to inactivate the glucose-oxidizing enzyme system of many varieties of organisms. To determine more closely the point of attack of the disinfectant, several of the enzymes involved in glucose oxidation were examined individually. Of these, triosephosphoric dehydrogenase alone was inactivated at the proper halogen levels, and this enzyme may therefore well be the one involved in the disinfection reaction. Bacterial spores, whose viability does not depend on the ability to oxidize glucose, were found to resist concentrations of halogen greater than those required to destroy the glucose-oxidizing system.

By a similar series of experiments it was shown at Columbia University that the action of organic mercurials could also be explained on the basis of an inhibition of triosephosphoric dehydrogenase and paralysis of glucose oxidation. However, this inhibition was reversible, whereas the action of the halogens was essentially irreversible. The mercurials apparently form complexes with the —SH groups of the enzyme, from which the mercury can be removed by other sulfur compounds such as cysteine or glutathione and the original activity restored. By contrast, halogenating reagents presumably oxidize the —SH groups of the enzyme in irreversible fashion.

Since the action of the most powerful known water-disinfecting agents seems to be as specific enzyme inhibitors, it appears that one should look to other inhibitors of key enzyme systems for new disinfecting agents.

MISCELLANEOUS

SURVIVAL OF ORGANISMS

As a background to the disinfection studies, the survival of the different types of pathogens in water under varying conditions of pH and pollution was determined. No decrease in numbers of *Esch. coli* over a one-hour period was observed between pH values of 4 and 11; *Eber. typhosa* showed no decrease between pH values of 5 and 10. Beyond these ranges hydrogen and hydroxyl ion exerted killing actions. Cysts of *E. histolytica* were not destroyed in two hours at pH values as low as 0.5 or as high as 13. At pH 14, killing took place in ten minutes. Studies on Theiler's virus showed that in tap water at 23° C. its potency was maintained completely for twenty-eight

days, but that in a polluted water (Charles River water) the infectivity began to decrease after thirteen days, and in 10 per cent sewage after nine days. *Leptospira icterohemorrhagiae* was found to survive at 25° C. and pH 7 for thirty days in sterile tap water, for eighteen days in air-contaminated tap water, for five to six days in Charles River water, and for three to four days in 10 per cent sewage. Survival in sterile tap water was reduced to 1.5 days at pH 5 and to three to four days at pH 8.5.

Disinfection of Canteen Lip and Neck

When the standard Army canteen is filled by dipping it in a source of water, the outside threads are exposed to contamination. Experiments showed that this area was not disinfected when the canteen cap was screwed home after the disinfecting tablet had been added to the water in the canteen. It was shown, however, that the lip and neck could be disinfected satisfactorily if the canteen cap was replaced loosely so that some treated water leaked out through it when the canteen was shaken after addition of the disinfecting tablet. By modifying the construction of the canteen slightly, as shown in the Final Report of the Harvard University group, it should be possible to ensure disinfection of the drinking surfaces without requiring special manipulation of the canteen cap.

Fruits and Vegetables

A problem corollary to the disinfection of water was the treatment of fresh fruits and vegetables with a disinfecting solution so that they might safely be eaten raw. Active free halogens are not suitable for this purpose, since they are quickly reduced by the organic material of the produce. Solutions of Mikroklene (active ingredients: azochloramid and Naccanol, an anionic detergent) at a concentration yielding 150 ppm of titrable chlorine, or solutions of Ceepryn, a cationic detergent, at a concentration of 50 ppm were found to destroy bacteria and cysts on infected produce within thirty minutes without impairing taste. Cysts could also be destroyed by immersing the produce for thirty minutes in water at 45° C. This did not cause the vegetables to lose their crispness.

Part Five: Chemical-Warfare Agents

INTRODUCTION

MILTON C. WINTERNITZ

MANY MONTHS have passed since the series of dramatic events leading to the end of World War II. They have been busy months, made difficult on various counts. There has been and still is indecision concerning the continuance of organizations and installations of proved worth for the war years and of promise for peacetime. There is a dearth of scientists; the supply was cut off at its source during the emergency, and now, with the mounting demands of scientific research, competition for the available personnel is more evident than co-operation between agencies of industry, education, and government, even though the advantages of working together have been demonstrated to be great. All these distractions have already caused the details of war experience to fade. Such enthusiasm as may be mustered for recording them can only be ascribed to the hope that advantage will accrue for charting future activities and avoiding past pitfalls.

The spring of 1917 witnessed a feverish effort to launch a defense against chemical warfare. On April 22, 1915, the Germans, after several futile efforts, had introduced gas as an effective weapon, and by 1917 they had developed the gas shell, with its increasingly menacing content.

The nucleus of what became the Chemical Warfare Service more than a year later was an emergency organization, sponsored by the Bureau of Mines of the Department of the Interior. At its center were the investigators versed in the control of toxic mine gases. Yandell Henderson was at the helm for the medical sciences. To him and to the Director of the Bureau of Mines must be credited the rapid development of knowledge concerning the defensive use of war gases. Moreover, loath as many were to join the enterprise — for the outcome could not possibly have been visualized — it must be admitted that this reaction was changed in the short year and a half of active

work. The effects of gas inhalation proved not only interesting but important — in a way that could not have been foreseen. Knowledge so gained clarified many problems both of structural and of functional alterations associated with pulmonary disease. The rapidly evolved organization was engrossed in a multitude of problems of promise when the Armistice was declared on November 11, 1918, and practically all investigation was promptly halted.

Two decades and more elapsed before the outbreak of World War II. In the meantime, the establishment of the National Research Council provided a mechanism for agencies of government to secure advice from the scientists of the country. The Committee on the Treatment of Gas Casualties was one of the later groups established by the Division of Medical Sciences of the Council on the request of the Surgeons General of the Army and Navy, concurred in by the Chief of the Chemical Warfare Service.

This committee was the first of a series of organizations. Its most important outgrowth was Division Five of the Committee on Medical Research. The chairmen of the various committees of the Division of Medical Sciences were appointed as consultants to the Committee on Medical Research promptly after its establishment with the National Defense Research Committee as one of the two divisions of the Office of Scientific Research and Development. By this mechanism the advisory function of these committees was extended to include the recommendation of contracts with universities, hospitals, and other agencies conducting medical research, made through the Committee on Medical Research to the Director of the Office of Scientific Research and Development. Intimate knowledge of progress of work under such approved contracts was essential both for arrangement of conferences between contractors and for advice concerning renewal of contracts. To meet these requirements, a more stable organization was necessary than could be provided through the occasional meeting of the members of the Committee on the Treatment of Gas Casualties with the authorized liaison officers from the services and also from co-operating nations.

Fortunately, the National Defense Research Committee was a well-organized outfit when its sibling, the Committee on Medical Research, came into existence in 1941, so that it was possible for the former's experience to be placed at the disposal of the Committee on the Treatment of Gas Casualties. A young medical scientist was secured to devote his full time to the business of the latter committee, with headquarters and secretarial assistance at the National Academy of Sciences. Here his contacts both with the officers of the National Research Council and with those of the Committee on Medical Research were frequent, so that he was informed of organizational policy and could attend meetings of committees when problems relating to war gas were under consideration. His location also facilitated the necessary contacts with the National Defense Research Committee and other agencies

of government, including particularly the offices of the Surgeons General of the Army, Navy, and United States Public Health Service, the Office of Civilian Defense, and the several divisions of the Chemical Warfare Service.

The facilities at Edgewood Arsenal in Maryland were promptly surveyed by the Committee and found disappointing in so far as they related to medical science. The quarters were entirely inadequate, and the personnel was not prepared either qualitatively or quantitatively to perform its duties. The over-all task was arbitrarily divided, and there was no cohesion among the various groups concerned with care of accidental injuries at the plant, toxicity studies in the Technical Division, investigation in the Division of Medical Research, and instruction at the School. Moreover, some procedures accepted on the basis of investigation were found to be hazardous.

The Committee on the Treatment of Gas Casualties proceeded to organize the approach to its assigned problem by dividing the field of investigation into its natural parts, including systemic and local effects following exposure to chemical-warfare agents, with further subdivisions to cover particularly ocular, dermal, and respiratory problems. Understanding of functional change was primarily sought — the reverse of the dominantly structural approach of 1917. A search was made for collaborators equipped to determine enzymologic and other biochemical effects, as well as pharmacodynamic actions and their correlations with structural change, as an approach to rational therapy. The results of such study are summarized in the chapters that follow.

Great advantages accrued through co-operation with the National Defense Research Committee and its component of exceptional chemists. They were ever ready to aid in securing essential compounds and to furnish essential advice. The differentiation of fields of work between the National Defense Research Committee and the Committee on the Treatment of Gas Casualties was clear except in the section of the former engaged in the study of physiological mechanisms, in which duplication of effort was avoided by frequent conference. Within the year, memorable in the first instance by the entry of the United States into the global conflict, the machinery of the Committee on the Treatment of Gas Casualties was well developed, operating, and producing.

In July 1942, on request of the British authorities, Colonel John R. Wood, newly appointed to represent chemical warfare in the Office of the Surgeon General of the Army, and the writer, who was the chairman of the Committee on the Treatment of Gas Casualties, embarked on a mission to Great Britain. We returned in one month, with a wealth of information accumulated by the Allies and placed at our disposal. The report of the mission covered studies of the following subjects:

(1) Air-raid precautions: instruction of civilian physicians in medical aspects of war gas; decontamination and cleansing centers and methods.

(2) Schools for training medical-service personnel, covering quick identification of chemical-warfare agents, protection of soldiers against gas attacks, and indoctrination of troops concerning chemical warfare, immediate self-treatment after contamination by a vesicant, and methods of handling casualties.

(3) Factories for large-scale production of chemical-warfare agents and their organization for the care of workers, including protection against toxic gases, first-aid treatment of ambulatory casualties, and the unique study of the nature and treatment of chemical injuries similar to those expected on the battlefield.

(4) Research laboratories associated with pilot plants, where methods for the mass production of diverse new agents were under investigation.

(5) The experimental station of the chemical-warfare establishment at Porton, in particular its special laboratories and quarters for experimental animals and volunteers.

(6) Special problems under investigation in civilian institutions. These embraced, besides the primary tasks, studies in aviation medicine, shock, and the value of penicillin as an antibiotic, together with the possibility of its mass production.

Among the impressions gained by the mission, the following may be of interest. Although Great Britain had been at war for more than three years, the viewpoint was optimistic and conditions were unusually good. A great opportunity was offered for close co-operation between this country and Great Britain, to their mutual advantage. Education in the problems of chemical warfare was far advanced, and intensive investigation in many branches was being pursued by an excellent group of scientists. New agents were being developed and their efficacy determined from many standpoints. The British view concerning gas was in many ways far in advance of ours. Some gases, like the tear and sneeze types, had been shown not only not to reduce efficiency seriously but to be readily adapted to. Troops were being taught to cleanse themselves of contaminants and to defend themselves against gas attacks. It was recognized that indoctrination in this field must be based on knowledge and on experience, and that the soldier must not be immobilized by protective gear or hampered by an unrealistic psychology of war.

The recommendations of the mission, all of which were promptly accepted and instituted, included closer liaison between American and British investigators; uniform symbols and methods of coding chemical-warfare agents; the assembly, filing, and correlation of literature and illustrations relating to medical aspects of war gases; and centralization at Edgewood Arsenal of investigation dealing with problems of medical science, in proximity to other major divisions of chemical-warfare study. The last two recommendations merit special consideration. The first of these constituted an

herculean task, which was splendidly executed under the direction of Dr. Levin Littleton Waters. The second was of outstanding importance, not only for the adequate development of the medical-science approach to the problem of chemical warfare and the constructive influence thus exerted on many other fundamental phases of the problem, but also for the many byproducts that have already proved to be of great value for man's health and well-being.

Dr. Waters's task fell into several divisions. Approximately six hundred illustrations, many in color, of the majority of the medical phases of gas warfare were assembled and made available to the services. Selected series with appropriate legends were required for teaching manuals; others were reproduced as strip film and used constantly for essential instruction of many different groups of service personnel and also of civilians. The originals, as well as the films, have been deposited for safekeeping at the Medical Research Laboratory at Edgewood.

The second part of this assembly was the preparation of a bibliography of chemical-warfare agents in their medical-science relationships. There were sixty separate reviews, and the resulting contributions have been assembled in three volumes dealing respectively with the eye, the respiratory tract, and the skin.

The centralization of investigation in the medical-science approach made at Edgewood was a most important undertaking. Several other primary fields of activity in chemical warfare were already established at the arsenal. There was a splendid new technical laboratory with a large complement of able chemists and a toxicologic subdivision. The school constituted a large plant for mass production of approved agents, and also pilot plants. The hospital included examples of chemical injury among its bed patients as well as among the ambulatory group. There were also proving grounds at the arsenal, and it was not only logical but highly desirable to develop the required facilities for biologic study in this environment. The major share of credit for this achievement belongs to Colonel John R. Wood, M.C., A.U.S., who labored for twelve months convincing authorities, preparing plans, and streamlining approach to essential building materials. On February 22, 1944, the commodious and efficient Medical Research Laboratory, with its attached facilities for housing many animal species, was dedicated.

The Medical Division of the Chemical Warfare Service was completely reorganized. The liaison between the Office of the Surgeon General of the Army and the Chemical Warfare Service was greatly strengthened by the induction of Dr. C. P. Rhoads into the Army with the rank of colonel, in charge of the Medical Division. He had been a member of the Committee on the Treatment of Gas Casualties, and its acting chairman while Colonel Wood and the Chairman were in Great Britain. He was well acquainted with the eighteen teams engaged in the various medical-science approaches

to the treatment of war-gas casualties, then under contract with the Office of Scientific Research and Development through the Committee on Medical Research, and had been in intimate contact with the investigators. This experience and the values accruing from it were continued and expanded by Colonel Rhoads in his capacity as Chief of the Medical Division, and have now become a pattern of promise in postwar organization of an important field of investigation, with which Colonel Rhoads became associated on his withdrawal from service in the summer of 1945.

Colonel Rhoads's influence for the development of essential co-operation through the senior council of the Chemical Warfare Service was great. It resulted in a much more efficient approach to many of the problems so evidently requiring attention, as indicated in the report of the mission to Great Britain. It extended further to the various great proving grounds that were soon developed and to the actual theaters of operation, all of which he visited.

In close co-operation with Colonel Wood, as Director of the Medical Research Laboratory, the task was undertaken of building up the essential personnel for its activities. The Committee on the Treatment of Gas Casualties, with the concurrence of the Committee on Medical Research, transferred to the laboratory many of the scientists included in its teams of investigation at the various universities throughout the country. As a consequence, at the end of the war, when the laboratory had already achieved distinction as an investigation center, only a few projects were left to be completed under the auspices of the Committee on the Treatment of Gas Casualties, by this time incorporated as part of Division Five of the Committee on Medical Research.

The organization of divisions for the Committee on Medical Research occurred in the early summer of 1944. Several of the various committees originally a part of the Division of Medical Sciences of the National Research Council had adopted the plan of technical aides, instituted after the fashion of the National Defense Research Committee organization. Many of the committees grouped themselves naturally into major fields like medicine, surgery, and so forth. Others, including those on insect control and rodent control, became part of Division Five.

Stimulated by General James S. Simmons of the Office of the Surgeon General of the Army, the Department of Agriculture undertook a search for ways of improved insect control as early as 1941. The facilities and personnel of several of the laboratories of the Bureau of Entomology and Plant Quarantine at Beltsville, Maryland, were assigned, and the station at Orlando, Florida, was expanded to meet requirements.

This advance was made possible by contracts involving repeated transfer of funds from the Committee on Medical Research to the Bureau of Entomology and Plant Quarantine. Old methods were compared with new ones

for the culture of the various insects under investigation, including several varieties of lice, flies, roaches, and mosquitoes. These preliminary steps were essential for determining the efficacy of compounds such as ovicides, larvicides, insecticides, and insect repellents. The compounds were gathered in increasing numbers with the aid of Division Nine of the National Defense Research Committee, and when leads developed new compounds were synthesized, again with the co-operation of this division.

It should be interjected that the testing of all these compounds, including those prepared for that specific purpose, was done largely on the basis of trial and error. There was no fundamental knowledge available for guidance concerning the relation between chemical structure and biologic action; indeed, insect physiology has admittedly lagged far behind similar mammalian study. When a compound of promise was disclosed, it became necessary to determine its toxicity and irritant qualities, both for various animal species and for man. This involved co-operation with the Food and Drug Division of the Federal Security Agency, facilitated, as were the investigations of the Bureau of Entomology and Plant Quarantine, by contract through the Committee on Medical Research. Assistance of high order also was available through the Laboratory of Industrial Hygiene of the United States Public Health Service and the Kettering Laboratory in Cincinnati.

All this was necessary, for it was essential to institute detailed study concerning the mechanism of action as well as the toxicity of the few compounds of promise that were being uncovered. Study of the solvents of such compounds was required, since many of these were in themselves found to be irritating or toxic even before incorporation of the specific compound. Such solvents were constantly being sought, for the use of compounds as powder admixed with a dry base proved to be limited. Application of powder or solution, as such or as an emulsion, varied in accordance with the objective sought.

Methods of dispersal of liquid preparations in accordance with requirements was another large task in which the National Defense Research Committee was actively co-operating. Hand equipment and various types of power equipment were being tried, and the latter involved motor conveyance by land, sea, and air. Here again the Office of Scientific Research and Development was concerned. Through its Office of Field Service, the essential contacts could be arranged for desired trials in theaters of operation.

Without doubt the impetus for expansion of this field of endeavor was greatly augmented by the rather accidental disclosure in the fall of 1942 of an insecticide that seemed to be the answer to the searchers' prayer. It was DDT. Like vitamins, it rapidly became a household name. Synthesized in 1870 by Othman Ziedler, a graduate student at the University of Strasbourg, it remained in obscurity for seventy years. Then its value as a moth-killer was demonstrated, but this was soon overshadowed when the usual impor-

tation of arsenic became impossible on account of war and DDT saved the
Swiss potato crop.

At Orlando, where it arrived unheralded for screening, the unrivaled
value of DDT was at once realized. Haller of the Beltsville laboratories de-
termined its structure and developed methods for its synthesis. This re-
sulted in an increasing production that reached more than 3,000,000 pounds
a month at the close of the war. Its uses for the war emergency multiplied
rapidly, and it reached a new peak of popularity in 1943, when by means of
DDT the typhus epidemic in Naples was abruptly stopped in midwinter, a
feat that had never before been accomplished.

A DDT Committee was set up in the Office of the Surgeon General of
the Army, with a component of subcommittees designed to cover all the
concerned fields of insect control, and with liaison representation from many
divisions of all services, including those of our allies. This committee also
considered problems of rodent control, for many of these species, as is well
known, are intermediary hosts of insect vectors of disease. The Office of
Scientific Research and Development was also concerned in this study, indeed
in ways that closely paralleled those involved by insect control. Contracts for
investigation in many directions were made with the Fish and Wildlife
Division of the Department of the Interior. They involved trial of new
compounds, many of which were made available by the National Defense
Research Committee, even in quantity essential for field testing. Here it
should be pointed out that the most valuable compound for the control of
rodents is a discard from the chemical-warfare pile, suggested with other
compounds to the Fish and Wildlife Division by the National Defense
Research Committee on the basis of extensive study in co-operation with the
Chemical Warfare Service.

Another compound, ANTU, developed under contract with the Com-
mittee on Medical Research, must be briefly noted. It was deliberately sought
by a psychologist, if you please, as the result of a unique experience that
emphasizes an inherent tendency of man uncontaminated by his milieu. A
child who ate salt pilfered from the pantry was found to have adrenocortical
insufficiency. She had instinctively saved her life by the procedure. The psy-
chologist repeated this episode with rats, and demonstrated that the rodent
was dependent on the taste buds in its tongue for selection of salt. Thus,
when later seeking a new rat poison, he searched for one that would be
acceptable because it was insoluble in the fluids of the mouth and conse-
quently tasteless. He found it, but unfortunately, valuable as it was for
control of the Norway rat, other rodents could eat many times the amount
with no ill effect.

There were also other projects under way through contract recommended
by the Committee on Medical Research, and involving various phases of
insect and rodent control.

All three divisions of the Office of Scientific Research and Development — the Office of Field Service, the National Defense Research Committee, and the Committee on Medical Research — were inextricably involved in the insect and rodent control problem. The experience of the preceding years indicated that the circumstances warranted creation of a committee for the correlation of work within this organization. This involved the broad general fields concerned with insect and rodent control and extended far beyond the province of any single agent. The Office of the Surgeon General of the Army concurred in this premise, urged the establishment of a committee as suggested, and promptly converted its DDT Committee into an Army Insect and Rodent Control Committee for the like correlation of its separate activities in the fields concerned and for co-operation with the similar committee of the Office of Scientific Research and Development. The latter committee was established by the Director on recommendation both of the Committee on Medical Research and of a special committee appointed to consider the problem. The committee, as finally constituted, included representatives from the three divisions of the Office of Scientific Research and Development, with invited liaison from the services and from agencies of government concerned with investigation of phases of insect and rodent control.

Five panels or subcommittees were authorized to cover the problems of chemistry, dispersal, entomology, biology, and rodent control. Stress was placed on special topics including the mechanism of action of DDT and of its isomers in varieties of insects and also in mammals, dangers of DDT intoxication and injury to man and other forms of life, treatment of DDT poisoning, solvents for and methods of its dispersal, and search for other insecticides and detailed study of any of promise. Emphasis was also placed on repellents, for none that met the desired requirements had been uncovered, on charting the distribution of rodents with particular reference to their part in the conveyance of disease, and on the comparison of available rodenticides and of various improved preparations and methods for their effective employment.

This organization was activated in September 1944. Technical aides facilitated the work of the subcommittee chairmen through contact with each other, the Army Insect and Rodent Control Committee, the Bureau of Medicine and Surgery of the Navy and its Research Division, the Laboratory of Industrial Hygiene of the National Institute of Health, the Food and Drug Administration of the Federal Security Agency, the Bureau of Entomology and Plant Quarantine of the Department of Agriculture, the Fish and Wildlife Service of the Department of the Interior, and the British Commonwealth Scientific Office. The technical aides were also intimately informed of work under way with the support of the National Defense Research Committee and the Committee on Medical Research, and this exchange of

information was materially increased as problems crystallized through conference and new groups were formed for desired undertakings.

In the course of the survey the parallelism of approach in the field of insect and rodent control and in the search for chemical-warfare agents became obvious. They both involved securing chemical compounds by hook or by crook. In the latter, toxicity for man was the desideratum; in the former, that for one or another species of insect or rodent. In both, the range of toxicity for many kinds of life had to be known; in both, knowledge of the mechanism of action and of therapy for possible accidental injury or poisoning and so forth was essential.

Knowledge concerning dispersal of chemical-warfare agents was far advanced and promised great assistance for the use of insecticides and the like. Naturally the possibility of adapting the Medical Research Laboratory at Edgewood, insofar as it was available, suggested itself. A plan was developed and concurred in enthusiastically by the Chief of the Chemical Warfare Service and his associates, including the Chief of the Medical Division and the Director of the Medical Research Laboratory. Personnel were secured, insectaria and equipment were installed, and the attack on physiological entomology was launched at Edgewood Arsenal.

One more task, and a vital one, of the Office of Scientific Research and Development committee was the development of the Co-ordination Center, described below. There was early recognition of the importance of insect and rodent control for peacetime, even though this was momentarily overshadowed by the requirements of the war. It was also clear that the Office of Scientific Research and Development was established by executive order to serve only during the emergency, and that other plans for a continuance of the Insect Control Committee would be necessary if, as was thought desirable, it should continue beyond June 30, 1946, when subsidy from the Office of Scientific Research and Development was to cease. These plans were consummated in the summer of 1945. With little change in organization, this committee was taken over by the National Research Council on combined request to the President of the Academy by the Director of the Office of Scientific Research and Development, the Secretaries of War, Navy, and the Interior, and the Director of the Federal Security Agency.

The Co-ordination Center, as it has developed, is almost entirely a monument to the industry and ability of Dr. C. Chester Stock. Starting as technical aide to the Committee on the Treatment of Gas Casualties in the summer of 1942, he had grown with the organization, and on his return to his prewar activities in February 1946, he had become Deputy Chief of Division Five of the Committee on Medical Research and Executive Secretary of the National Research Council's Insect and Rodent Control Committee.

With the assistance of the technical aides of the various subcommittees and

with an adequate secretarial staff under executive direction in commodious quarters, there were assembled background and current reports on pertinent results in the various fields. These reports were summarized, and when significant information had accumulated on any topic a review was prepared. This work has been completed for many subjects.

The technical aides early conceived the plan for a bulletin summarizing individual reports of investigators as well as many reports from current scientific periodicals, including some of primary interest to industry. This bulletin has appeared biweekly and is now in its thirtieth edition, comprising a total of eight hundred pages. The content is divided for convenience into major fields to conform with subcommittee organization, and the two volumes now completed, each representing a six-month period, have detailed indices. Until recently each edition of the bulletin, numbering approximately six hundred copies, has been divided, with limitation of circulation of highly classified information. As declassification has progressed it has been possible to increase the size of editions. The last two, numbering fifteen hundred copies each, have been widely circulated among educational and industrial laboratories.

A unique feature of this bulletin is the fact that it brings its briefly summarized content to all the scientists interested in the problems of insect and rodent control and serves to orient them in the advances of the whole field, often acquainting them with facts that might not otherwise have come to their notice. This information has repeatedly proved to be of particular significance in the light of individual experience. In this way the bulletin continues the advantages of conference found so valuable during the war. That such dissemination of knowledge may have advantages for groups whose efforts are directed toward the same objective through different approaches requires no emphasis. That an apparently trivial objective may be highly significant for the solution of an even more important problem, and that this may be recognized and utilized by a member of such a conference group, cannot be doubted. A few examples will emphasize this fact.

BAL, a dithiol propanol, was developed for the treatment of an arsenic containing so called "blister" war gas. It was shown to be able to compete successfully against protoplasm for arsenic in both unicellular animals and mammals. It is being used effectively in the therapy of systemic arsenic poisoning in man and of poisoning with several other varieties of heavy metals, including mercury.

Another war gas discarded for lack of toxicity to higher animals was found to be highly toxic for rodents. Its nonvolatile sodium salt is now the best available rodenticide.

A third discarded war gas, one of a large series with similar tendency, is selectively toxic to particular groups of cells of undifferentiated varieties. It is proving to have great value in unraveling problems of biology, but it

may also have its place side by side with radiotherapy in the treatment of particular forms of malignancy. Evidence of temporary beneficial results in the treatment of lymphoma in mice led to its cautious use in man. Results so far obtained indicate that it is not a cure. The tumors recur, as these varieties do after radiotherapy, but like radiotherapy the compound will probably be valuable in ameliorating symptoms and extending comfortable and useful existence.

Seizures quite indistinguishable from convulsions characteristic of epilepsy may be produced with still another compound. Its study has disclosed fundamental facts concerning the conversion of pyruvate to glucose, and this may be a factor in the serious variant of cardiac action called fibrillation. Mention should also be made of a compound that inhibits the enzyme cholinesterase with symptomatic improvement in several serious diseases. Effective therapy may result from this lead.

These are only some of the facts that have been uncovered, and the truth of the time-worn quotation, "What's sauce for the goose is (not always) gravy for the gander," becomes increasingly evident. Serendipity may not always be the result of the same degree of chance. The prepared mind and organization to facilitate its exposure cannot be ignored.

In retrospect it is hoped this may have been a consideration when another function of the Information Center was initiated. Like many other organizations, the Insect and Rodent Control Committee accumulated a vast store of data concerning chemical compounds and their biologic action. The very nature of the investigation required systematization of this information, in order to avoid duplication of effort on the part of various groups engaged in the work; to supply individual investigators with specific knowledge concerning availability of particular compounds and details of their structure and properties, toxicity, and more definitive biologic action; and, finally, to provide the scaffolding for the scientific as distinct from the more empirical approach to the problem under investigation.

To facilitate access to the accumulating factual information, a card-index system was established. Each of the thousands of chemical compounds has a card containing details of its structure, availability, and important biologic characteristics as determined frequently on many forms of life, including both plants and animals. The latter category covers mammals, even man, as well as insects and rodents. A study was instituted to ascertain how this index system could attain maximal usefulness, and it now seems both possible and practical to utilize the well-known punch card and mechanical sorting system for the rapid separation of both the particular chemical structure desired and its specific biologic action, in so far as these have been determined. This technical procedure, it is believed, will be invaluable in making readily available the extensive information concerning chemical structure and biologic action of compounds. The number of these compounds, already

large, has been rapidly increased during the intensive search of the last few years for insecticides and insect repellents, for chemical-warfare candidates, and also for effective chemotherapy for many disease-producing or material-destroying agents.

The mechanism is now established for the reception of compounds constantly being prepared in the laboratories of the educational and industrial institutions of this country. At the Co-ordination Center at the National Academy of Sciences, the factual information concerning their chemical and physical properties can be documented. The accumulated information available to the chemical committees for comparison will aid in further distribution of compounds for determination of effects on many forms of life, including plants as well as animals and encompassing the whole gamut from virus to man. Further studies designed to ascertain the mechanism of the action of selected compounds may be undertaken if the Committee continues.

CHAPTER XXXVI

SYSTEMIC AGENTS: ACTION AND TREAT-MENT

ALFRED GILMAN AND MCKEEN CATTELL

O NE OF THE striking departures in chemical-warfare research during World War II was the emphasis placed on systemic agents. Not only were the systemic effects of the vesicants appreciated for the first time, but also a search was continuously pursued for highly toxic compounds that could exert a rapid lethal action following absorption, either from the lungs or from the skin.

The more promising of the systemic agents received careful scrutiny. These studies had as their main objective the determination of toxicity, but they also included fundamental investigations of mechanisms of action in order to define measures of therapy more clearly and to understand better the processes of toxicity. Moreover, in some instances the search for antidotes for specific gases was the focal point of a program in which a large number of compounds were synthesized for the purpose of finding outstanding antagonists. From the point of view of the medical interest in chemical warfare this approach has yielded unsuspected and fruitful byproducts. In the course of investigations on these agents not only have valuable research tools for the solution of many of the problems of cellular physiology and metabolism been uncovered, but also many potential therapeutic agents have appeared.

It is not possible in a limited review to cover all the compounds investigated as potential systemic poisons, nor would such an exhaustive discussion be of general interest. Instead, the essence of the investigation of groups of agents that excited the most attention and have contributed to fundamental medical concepts will be reviewed. These are the nitrogen and sulfur mustards, fluoroacetates, cyanides, British anti-lewisite (BAL), and fluorophosphates.
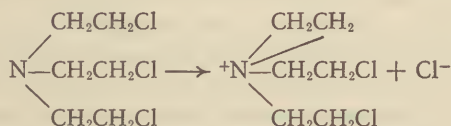
THE NITROGEN AND SULFUR MUSTARDS

At the conclusion of World War I, it was generally believed that mustard gas exerted its vesicant action by releasing hydrochloric acid intracellularly. Only a few isolated reports pointed to the fact that patients severely poisoned with mustard gas were prone to develop leukopenia and damaged bone marrow. Investigations of mustard were dormant following World War I, and little was added to the inadequate understanding of the so-called "King of the Battle Gases." In the decade between 1930 and 1940, chemists in the United States, Great Britain, and Germany reported the synthesis of the nitrogen analogues of mustard; namely, bis and tris beta-chloroethyl amines. These analogues were shown to be vesicant in much the same manner as mustard itself, and the action was attributed to the beta-chloroethyl group in its attachment either to nitrogen or to sulfur. Naturally these compounds were considered as potential chemical-warfare agents. With the advent of World War II and the knowledge that the Germans had synthesized large amounts of the nitrogen mustards, research on this type of compound was greatly intensified.

It was early appreciated that the nitrogen mustards exerted a more significant systemic toxic action than did mustard gas itself. Not only could the compounds penetrate to the circulation by way of the lungs, but also absorption could occur to a significant degree through the skin. Indeed, were this series of agents to be extensively used as vesicants, it was expected that a significant number of casualties from the systemic effects would result. As a consequence, the mechanisms of systemic toxicity received close scrutiny.
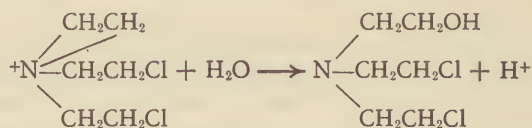
Chemical Reactivity of the Mustards

At the time of the synthesis of the beta-chloroethyl amines it was appreciated that compounds of this type would undergo cyclization in aqueous solution and thus be changed from tertiary amines to quaternary imonium bases. This reaction for tris beta-chloroethyl amine is as follows:

$$\begin{array}{ccc} & CH_2CH_2Cl & CH_2CH_2 \\ & / & / \\ N & -CH_2CH_2Cl \longrightarrow {}^+N{=}CH_2CH_2Cl + Cl^- \\ & \backslash & \backslash \\ & CH_2CH_2Cl & CH_2CH_2Cl \end{array}$$
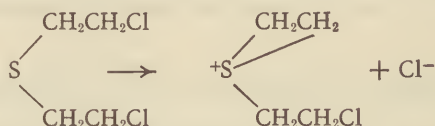
Whereas the original tertiary amine is a relatively nonreactive product and, indeed, except for the property of cyclization may be said to be chemically inert, the quaternary imonium compound with its ethylenimonium ring is

one of the most reactive of organic chemicals. So reactive is it that reaction occurs readily even with water, in the following manner:

$$\begin{matrix} CH_2CH_2 \\ {}^+N{=}CH_2CH_2Cl + H_2O \longrightarrow \\ CH_2CH_2Cl \end{matrix} \begin{matrix} CH_2CH_2OH \\ N{-}CH_2CH_2Cl + H^+ \\ CH_2CH_2Cl \end{matrix}$$

If substances other than water are present they can react competitively. So reactive are some compounds that in their presence reaction with water is negligible. As examples of compounds of biologic importance that readily react with the ethylenimonium ring of the nitrogen mustards may be mentioned the a-amino, imidazole, sulfide, phenolic, ϵ-amino, and imino groups of amino acids and peptides; inorganic phosphate, as well as glycerophosphate and hexose phosphates; the amino groups of adenosine and thiamine; and the pyridino-N of nicotinic acid amide and pyridoxine. In addition, reactions have been demonstrated to occur with proteins such as hemoglobin, insulin, gelatin, crystalline egg albumin, tobacco mosaic virus, ovalbumin, and protamine, as well as various purified enzymes. Thus it appears likely that the basis of the systemic toxicity of the nitrogen mustards resides in the high chemical reactivity of the ethylenimonium ring.

The information afforded the chemists by the nitrogen mustards was the necessary tool for the elucidation of the chemistry of the sulfur mustards. Thus, in aqueous solutions sulfur mustard readily forms a sulfonium ring as follows:

$$\begin{matrix} CH_2CH_2Cl \\ S \longrightarrow \\ CH_2CH_2Cl \end{matrix} \begin{matrix} CH_2CH_2 \\ {}^+S \\ CH_2CH_2Cl \end{matrix} + Cl^-$$

The ethylenesulfonium ring is even more reactive than the ethylenimonium.

Systemic Toxicity and Pathology

The outstanding systemic action of both the nitrogen and sulfur mustards is that which causes, in a manner still unexplained, the death of cells. Of outstanding interest is the fact that cells vary in their susceptibility to the mustards and that this variation appears to be related to their proliferative activity. Thus, following systemic administration to experimental animals dissolution of lymphoid tissue is the earliest effect to be noted; other vulnerable tissues include the bone marrow and the mucosa of the intestinal tract. Pathological examination of animals poisoned by the nitrogen mustards reveals the following. An intestinal lesion is prominent which progresses from vacuolization and nuclear swelling of the epithelial cells to even-

tual necrosis and desquamation with hemorrhage. Lymphoid tissue throughout the body is uniformly involved. Lymphatic fragmentation may be evident within ten hours, leading to a persistent lymphatic atrophy for a number of days. In bone marrow early changes include swelling and alteration in the staining reaction of hematopoietic cells and a disappearance of mitotic activity. Progressive depletion of the marrow follows, and eventually almost complete aplasia results. The clinical course preceding death from nitrogen mustard poisoning is characterized by nausea and vomiting, severe diarrhea, which may become bloody, progressive emaciation and dehydration, and collapse.

Possible Mechanisms of Cytotoxic Action

Inquiries regarding the fundamental mechanisms by which actively proliferating cells are poisoned by the mustards have revealed provocative facts. Attention was early focused on the effects of the mustards on cellular metabolism and cellular enzyme systems. It was soon learned that diverse cells and tissues subjected to the toxic effects of the mustards evidenced marked metabolic defects. This led to the theory that the primary mechanism of action of the vesicants is the inactivation of essential cellular enzymes. As a result, the sensitivity of a large number of enzyme systems to the mustards has been determined. The majority of these proved only moderately sensitive or even resistant to inactivation. Among the highly sensitive enzyme systems were hexokinase, as well as creatine and pyruvate phosphokinases, inorganic pyrophosphatase, adenylic acid deaminase, and choline oxidase. This discovery has led to the theory that the basic mechanism of cytotoxic action may be the result of the action of the mustards on the phosphokinases. However, doubt is cast on this theory for the reason that there is no adequate correlation between the effects of the mustards in vivo and in vitro, and that many of the nuclear actions of the mustards can be elicited by concentrations having little effect on metabolic processes.

It is the actions of the mustards on cell nuclei that are the most provocative of the systemic effects of these compounds. It has been shown that the mitotic activity of a variety of cells from representative unicellular invertebrate, amphibian, and mammalian organisms is peculiarly sensitive to inhibition by minimal effective doses. For example, mild exposure of yeast cultures to sulfur mustard can produce an immediate reduction in growth rate, which may be sustained at reduced levels by several succeeding generations of daughter cells before recovery is apparent. Similarly, the early cleavage of the sea-urchin egg is inhibited or retarded by brief immersion of either the unfertilized egg or the early zygote in minimally effective concentrations of the mustards. The exposure of young salamander larvae elicits an immediate cessation of growth, which can be attributed to an inhibition of mitotic activity in the proliferating regions of all the embryonic tissues. The cells

in which mitotic activity has been completed at the time of exposure continue functional differentiation in a normal manner. Following direct application of threshold amounts of the mustards to the intact eye or after parenteral administration of minimal lethal doses, the corneal epithelium of mammals can be largely depleted of mitotic figures for a period of several days without visible evidence of concomitant cytoplasmic or nuclear damage. Moreover, for several days after the parenteral administration of doses sufficient to cause lymphopenia and granulocytopenia in rats, mitotic activity is decreased in lymphoid, myeloid, and erythroid cells of hematopoietic tissues that have escaped the initial destruction caused by the agents, as well as in the intestinal mucosa. Finally, the mitotic rate of regenerating cells following partial hepatectomy has been found to be significantly lowered by the intravenous injection of sulfur mustard. In this regard it is important to note that similar doses do not evoke visible pathologic changes in normal, nonproliferative hepatic tissue.

The inhibition of mitosis caused by mild exposure to the mustards does not in itself imply a primary nucleotoxic action of the agents. In fact, the mitotic arrest appears to be confined to the resting phase of the mitotic cycle. Cells in active mitosis at the time of exposure complete their division, with the result that the inhibited tissue may ultimately become depleted of mitotic figures. However, evidence of a more direct toxic action on nuclear mechanisms is furnished by the appearance of extensive nuclear fragmentation in cells of the corneal epithelium that have been exposed to doses somewhat higher than those that effect only a mitotic inhibition. The nuclear fragmentation and resultant chaotic chromatin dispersal can be considered as a pathologic and incomplete mitosis.

More convincing for the association of mitotic arrest with primary nuclear damage were studies on the inhibition of mitosis of pollen grains following exposure of Tradescantia inflorescences to minimal concentrations of sulfur mustard. The fate of the treated cells was shown to vary with the extent to which chromosomal abnormalities were elicited. Thus, severe exposure in association with complete mitotic arrest caused multiple chromosome breaks, resulting in fragmentation, pycnosis, and ultimately death of the cell. Mild exposure, which prolonged the resting period of the pollen grains, caused chromosomal breaks in many of these cells. If these were not too numerous or were followed by translocation, they were transmitted to daughter cells in the subsequent mitosis as hereditable chromosome abnormalities.

Perhaps the most significant demonstration of specific nucleotoxic action has been obtained from observations of the profound disturbances produced by the mustards on the structure and function of chromosomes in *Drosophila melanogaster*. Exposure of both male and female adults to sublethal doses was found to reduce or suppress fertility through disturbances of miosis and

mitosis in the gametogenesis of both sexes. However, following exposure of adult males to lower doses, which did not unduly reduce fertility, the genetic analysis of the x-chromosomes revealed a high incidence of sex-linked lethals greatly in excess of the natural rate of mutation, as well as a significant number of translocations and inversions. No other class of chemical agents has been shown to have such specificity of action on chromosomal mechanisms. Indeed, in the past similar effects have only been attained to the same degree by the use of short-wave radiation (x-ray and ultraviolet).

Clinical Applications

The marked effects of the mustards on lymphoid tissue, coupled with the finding that actively proliferating cells are selectively vulnerable to the cytotoxic action of the mustards, suggested the therapeutic use of these compounds in the treatment of neoplasms of lymphoid tissue. Because of its undesirable physical properties and extreme chemical reactivity sulfur mustard does not lend itself to parenteral administration, but nitrogen mustards in the form of their hydrochloride salts are water-soluble crystalline compounds, which can be readily dissolved in sterile saline solution for intravenous administration. Experiments on transplanted lymphosarcomas in mice revealed that dissolution of such tumors could be rapidly effected, although the dose required bordered on the toxic and the tumor invariably returned. The first clinical trial of the nitrogen mustards was conducted on a group of 6 patients in the terminal stages of various neoplastic diseases. In 2 cases of lymphosarcoma in which x-ray therapy had been discontinued, a rapid dissolution of large tumor masses followed a course of injections. The results were sufficiently encouraging to warrant further clinical experimentation.

To date approximately 150 patients have been treated by several groups of investigators. For the most part observations have been limited to selected cases of Hodgkin's disease, lymphosarcoma, and leukemia. The findings may be summarized in general terms. The most favorable effects have been obtained in patients with Hodgkin's disease. Remissions characteristic of those that follow careful x-ray therapy have been observed. Symptoms were quickly alleviated and physical evidence of lymphadenopathy, splenomegaly, and hepatomegaly regressed. It was necessary to repeat the treatment at intervals varying from one to eight months. Less favorable results have been obtained in cases of lymphosarcoma. The response in acute and chronic lymphogenous and myelogenous leukemias has been disappointing.

The action of the available nitrogen mustards on lymphoid tissue has not yet reached the degree of specificity that precludes undesirable action on the hematopoietic system. At present dosage is limited by the occurrence of moderate granulocytopenia, thrombocytopenia, and anemia. However, following a series of four injections of 0.1 mg./kg. an adequate clinical

response is usually obtained without affecting the formed elements of the blood to a serious degree. In addition, nausea and vomiting are very likely to occur for a brief period after each injection. No other undesirable effects on the gastrointestinal tract have been observed.

Although some patients receiving nitrogen mustards have been followed for a period of twenty-eight months, the evaluation of the clinical status of this group of compounds will require many more years of careful study. At present there is no basis for assuming that the therapeutic efficacy of the nitrogen mustards is any greater than that of x-rays.

It is possible that the potential value of the nitrogen mustards in the treatment of neoplastic diseases will be fully realized only when the opportunity to explore the relationship between chemical constitution and pharmacodynamic action has been exhausted. At present only two of the nitrogen mustards have been investigated clinically; namely, bis beta-chloroethyl methyl amine and tris beta-chloroethyl amine. These have been the products of a screening program designed for the evaluation of toxic chemical-warfare agents rather than of compounds of therapeutic interest. Literally hundreds of congeners remain to be synthesized and evaluated. Thus a series of compounds that can reproduce in many ways the cellular effects of x-rays is available for chemical and biologic investigation. It is to be hoped that the previous successes that have characterized the evolution of chemotherapeutic agents by chemical alteration of a parent compound may be duplicated in the case of the beta-chloroethyl amines. The result would be a compound having a sufficiently specific toxic action for certain types of proliferative cells to possess therapeutic value.

FLUOROACETATES

The fluoroacetates were first investigated as potential chemical-warfare agents by British scientists, who learned of their toxicity from Polish reports. The compounds proved of outstanding interest, and although they did not fulfill all the requirements of a systemic chemical-warfare agent, the efforts expended on their study have been more than repaid by the fact that sodium fluoroacetate has proved to be the most outstanding all-purpose rodenticide.

Fluoroacetates comprise a wide variety of chemically related compounds. These compounds share many actions that have been attributed to the toxicity of the fluoroacetate ion. It was first thought that the toxicity of this ion would be similar to that of the well-known iodoacetate. In the case of fluoroacetate, however, the bond between fluorine and carbon is so firm that the ion shares none of the chemical and pharmacologic properties of the other halogenated acetates.

Fluoroacetate is unusual in its pharmacologic action in that species vary

widely not only in their susceptibility to the ion but also with respect to the type of response that they exhibit. Thus, lethal doses vary one hundred fold between different species, and the site of action may be either predominantly the nervous system or the myocardium. The most susceptible of all species is the dog. Severe convulsions and ultimate death result from the intravenous administration of as little as 0.1 mg./kg. At the opposite end of the susceptibility scale are various species of primate (including man), in which the lethal dose lies between 5 and 15 mg. Had the order of susceptibility been reversed, fluoroacetate would have represented by far the most outstanding systemic toxic agent, for the high vapor pressure of such compounds as methyl fluoroacetate and fluoroethanol would have permitted the attainment of field concentrations that were lethal following a single inhalation.

The fundamental contributions that the fluoroacetates can eventually make to medical science will result from the correlation of the basic mechanisms of action on cells with the effects on a given organ. Pertinent data have already been obtained as a result of research during the war years, and there is every reason to believe that the fluoroacetates will represent prominent and valued research tools.

As mentioned above, these compounds affect either the nervous system or the heart. In species susceptible to the action on the central nervous system, fluoroacetate is a powerful convulsant, and electroencephalographic analysis of the brain-wave pattern produced by this ion reveals a striking similarity to that of petit mal. The species highly susceptible to the convulsant action are the dog, cat, pig, and guinea pig; of these, the cat and pig also show serious cardiac involvement. The actions of fluoroacetate on the heart are even more striking. Outstanding is the development of a pulsus alternans, which is manifested by alternation in the heart sound and force of contraction, with the ultimate development of a 50 per cent pulse deficit. In the electrocardiogram this is reflected by alternation in the T-wave, which may be so extreme that alternate complexes are positive and negative. Early in the course of poisoning ectopic beats may be observed, which become more and more frequent and arise from multiple foci. Eventually, one of these beats arises during the vulnerable period and ventricular fibrillation results. The rabbit, goat, and horse invariably die of ventricular fibrillation and show no evidence of action on the nervous system. The various primates studied also succumb to the cardiac effects, but may exhibit mild, brief epileptiform convulsions before the onset of ventricular fibrillation. There is every reason to believe that death in human beings would result from the cardiac action of fluoroacetate.

In searching for the explanation of a basic mechanism of action of the fluoroacetates, attention has been directed to their effects on the intermediary metabolism of carbohydrates. It has been found that the agent inhibits specifically the oxidative metabolism of certain intermediates of carbohydrate

breakdown. Provocative is the suggestion that the specific action of fluoro-acetate is to block the oxidation of acetate itself by competitive inhibition of the enzyme systems on which the utilization of acetate is dependent.

Should future research demonstrate that fluoroacetate is a specific inhibitor of acetate metabolism — and evidence obtained during the war years points strongly to this conclusion — a research tool will have been afforded that may greatly further the understanding of this difficult field of intermediary metabolism. Moreover, there are the immediate implications of the obligate dependence of certain tissues on this simplest of substrates. It is known that the isolated heart can be readily maintained with acetate as the only oxi-dizable substrate. The specific cardiac actions of fluoroacetate suggest that the myocardium not only can utilize acetate as a source of energy but is also uniquely dependent on it. Likewise, in certain species the same applies to nervous tissue.

The outstanding successes scored by sodium fluoroacetate as a pesticide have been reported elsewhere. Thus, while fluoroacetate failed to qualify as a chemical-warfare agent, two fruitful byproducts have resulted from its in-vestigation. The conservationists and epidemiologists have access to a com-pound unsurpassed in its field, while the biochemist, physiologist, and pharmacologist have gained a research tool that may form the basis for new fundamental concepts of cellular metabolism.

CYANIDES

During World War I hydrocyanic acid was considered to be a potential chemical-warfare agent, but the weapons available at that time were in-effective in establishing adequate concentrations. During World War II, however, the gas was viewed much more favorably owing to the superiority of the available methods of dispersal. Included with hydrocyanic acid was cyanogen chloride.

From the medical point of view there were two problems regarding the cyanides that were of general interest. The first was concerned with the fact that the first breath of cyanide-contaminated air stimulated the chemorecep-tors of the carotid body to such a degree that the ability voluntarily to stop respiration while adjusting the gas mask was questionable. The second prob-lem was the treatment of cyanide poisoning.

The classical observation that methemoglobin can combine with and thus effectively remove cyanide from its combination with cytochrome oxidase was amply confirmed. The fact that animals could be saved from cyanide poisoning by adequate treatment with nitrites and sodium thiosulfate was also demonstrated. However, it was extremely doubtful whether therapy could be administered under field conditions, owing to the necessity of im-mediate treatment. Attention was therefore directed toward the possible

practical value of inducing a chronic, prophylactic methemoglobinemia in the event that this war gas was employed.

The best agent for the production of methemoglobinemia was found to be p-amino-propiophenone. The oral ingestion of 2.0 mg./kg. produced a methemoglobinemia of 20 to 30 per cent. The question of whether a significant concentration of methemoglobinemia could be maintained in the blood over extensive periods was also investigated. A 20 to 30 per cent methemoglobinemia maintained over a period of seven days had no effect on renal function, but at the end of this period there were observed the beginnings of a hemolytic anemia.

Exercise-tolerance tests were performed to define the limit of methemoglobinemia compatible with efficient work output. It was found that with concentrations of methemoglobin up to 15 per cent of the total hemoglobin there was no interference with light work loads. On the basis of animal experiments such concentrations would have protected against ten lethal doses of cyanide and from observations in human beings would have prevented any significant effects on the carotid body. Another interesting observation was the finding that a 30 per cent methemoglobinemia had no effect on dark adaptation.

Cyanogen chloride was found to be an agent that not only possessed the systemic toxicity of cyanide but was also a pulmonary irritant. Low concentrations of methemoglobin in the blood protected against the systemic effects but could not prevent pulmonary edema.

The results of studies on cyanide as a potential chemical-warfare agent have contributed greatly to our knowledge of methods of treatment of poisoning from this ion, which should prove of value for future application.

BAL (BRITISH ANTI-LEWISITE)

Among the various new agents developed under the stimulus of war necessity one of the most important from both a practical and a theoretical standpoint is BAL. This compound had originally been developed in England, and in a series of publications from Peters's laboratory at Oxford its effectiveness in protecting tissues against damage from lewisite and other organic arsenicals was demonstrated. BAL is 2,3-dimercaptopropanol, one of many dithiols investigated; its code name, given in this country, is derived from the term British Anti-Lewisite.

Long before the discovery of the effectiveness of the dithiols as protective agents against the toxic action of various metal salts, it had been demonstrated by Voegtlin et al. and confirmed in several laboratories that various monothiols such as reduced glutathione and thioglycolic acid counteract the toxic action of arsenoxide on both trypanosomes and mammalian species. The mechanism of this protection is believed to be one of competition; that

is, the SH groups of the protective agent compete for the arsenic that would otherwise combine with similar groups in the body proteins. However, when the monothiols were tested against lewisite they were found to be quite ineffective, and it was this fact and the theoretical consideration that the dithiols might form relatively stable ring compounds with trivalent arsenicals that led to the preparation and testing of dithiols by the British investigators. Not only was BAL shown to be effective in protecting the pyruvate-oxidase system in studies in vitro, but local application to lewisite skin burns in animals was a life-saving measure.

An intensive program of research on BAL and on related compounds was organized in this country under the auspices of the Committee on Medical Research and the National Defense Research Committee. The work reviewed in this chapter was done under direction of the Committee on the Treatment of Gas Casualties, Division of Medical Sciences of the National Research Council. Emphasis was placed on the development and testing of new compounds related to BAL in the hope of improving therapy; on the development of practical methods for the utilization of BAL in therapy, both by local application and by systemic administration; and on the pharmacologic and toxic actions of BAL, with a view to obtaining knowledge of its dangers and the procedures to be employed in case of overdosage.

PHARMACOLOGY

Extensive pharmacologic investigations utilizing various species of animals have contributed much information on the toxic hazards of BAL and the mechanisms of its actions in the living animal. The average fatal dose given by vein at one time to cats is approximately 0.03 cc./kg., an amount far in excess of that effective in the treatment of arsenic poisoning. Small doses cause lacrimation, blinking of the eyes, and salivation. With larger doses these symptoms are followed by blepharospasm, edema of the conjunctiva, an ataxic gait, and failure to respond to an ordinarily painful stimulus. Recovery is complete within four or five hours. When fatal doses are given, all these symptoms become severe, and there are gasping, rapid respiration with frothy exudate from the lungs and convulsions of the myoclonic type. The effects of BAL described above refer to the cat, but other species exhibit the same signs to a varying degree.

An analysis of the pharmacologic mechanisms concerned shows that effects on the circulatory system are prominent and probably play a major role in the fatal outcome from large doses. Very small doses of BAL result in a gradual increase in the arterial pressure, but larger doses have the opposite effect. When it is given by vein there is a sharp drop in the blood pressure. Recovery is rapid, but if the dose is sufficiently large this is followed by a slow decline to shock levels over a period of several hours. These effects re-

ceive their explanation on the basis of two prominent actions of BAL, a pronounced constriction of the peripheral vasculature and a marked increase in capillary permeability.

The peripheral vascular constriction following an intravenous dose of BAL is greater than that reported for any other drug, and is so extreme that the flow of blood through the hind limbs may be completely arrested. This affords an adequate explanation for the rise in arterial pressure observed with small doses, but since the peripheral constriction is also maintained during the period of falling pressure it is evident that other actions are involved. The fact that the vessels in the splanchnic area do not share in the constriction and the demonstration of a marked rise in portal pressure suggest that a redistribution of blood may be partly responsible for the decline in arterial pressure, but a more potent factor is probably a direct action on the capillaries.

Capillary damage resembling that occurring in peripheral vascular failure is a prominent feature in advanced BAL poisoning. This is evidenced in a several-fold increase in lymph flow from the thoracic and cervical ducts and, in animals given an intravenous injection of a nondiffusible dye, by a deep staining of tissues. Loss of plasma from the circulating blood is also reflected in the hematocrit readings. It is this shocklike state that appears to be the immediate cause of death, and this in turn is undoubtedly mediated through some disturbance in cellular metabolism.

Various changes indicative of disturbed metabolic function are seen in the blood and tissues of the intact animal following the administration of toxic amounts of BAL. These include a reduction in the pH of the blood, a decrease in the carbon dioxide combining power, an accumulation of lactic acid, and hyperglycemia. There is a depletion of glycogen and potassium in the liver. No consistent morphologic changes have been reported, even when BAL is given repeatedly over a period of weeks.

BAL is a strong reducing agent and interferes with cytochrome oxidase activity by keeping cytochrome in a reduced state; it interferes with oxidation in isolated tissues and exerts an inhibitory action on glycolysis in brain slices. It is probable that the toxic effects following the administration of large doses to animals are a reflection of actions such as these on essential enzyme systems.

Some of the pharmacologic actions of BAL described in animals have been observed in man following the administration of large doses — 0.005 to 0.008 cc./kg. — by muscle. Both the systolic and diastolic arterial pressures are increased and the heart is accelerated. The patient may complain of various paresthesias, including tingling of the nose, eyes, mouth, and skin. Aching pain referred to various regions of the body is a common symptom, as is a sense of warmth. Other signs and symptoms observed are perspiration, lacrimation, blepharospasm, salivation, vomiting, unrest, apprehension, weak-

ness, and fatigue. The effects develop soon after the injection and subside quickly, usually within an hour.

BAL is extremely irritating to the mucous membranes, and when applied in full strength to the skin it causes erythema and edema. In dilutions of 5 to 10 per cent, which are therapeutically effective, BAL may be applied to the eye or administered by intramuscular injection with only insignificant irritation.

ACTION IN SYSTEMIC POISONING BY METAL SALTS

Following the important studies of Bunting and Durlacher, in which it was shown that it was not necessary to decontaminate directly or neutralize lewisite applied to the skin in order to protect against the systemic effects, but that BAL was effective when applied to skin areas distant from the site of the lewisite burn, emphasis was directed toward the investigation of the therapeutic applications of systemically administered BAL. It was soon established that the treatment of systemic arsenic poisoning was more effective when the BAL was injected than when it was applied locally. The optimum therapeutic results are secured when the molar ratio of BAL is at least three times that of the lewisite applied to the skin. Animals that have been given more than a fatal dose of lewisite require amounts of BAL that produce toxic effects, and the best results are then obtained by giving it in divided doses. Striking improvement of symptoms and the saving of life have been reported even when the BAL treatment is delayed until after the development of pulmonary edema.

The application of BAL to the treatment of the toxic actions of the trivalent arsenic employed in the treatment of syphilis has received much attention. In experimental Mapharsen poisoning in rabbits and cats BAL is effective in saving animals from doses that would otherwise kill them all. There is every reason to believe that the therapeutic effectiveness of BAL is due to a direct reaction with the arsenic, thus removing it from its site of action in the body proteins. An insoluble thioarsenite complex is formed that is comparatively nontoxic. The resulting increased elimination of arsenic in the urine was first noted in England in rats poisoned with lewisite and was studied in detail in rabbits, cats, and man poisoned with Mapharsen in the Johns Hopkins and Cornell laboratories. In all these species a several-fold increase in the rate of urinary excretion of arsenic follows the administration of BAL, the effects of a single injection lasting for about four hours. In cats it has been shown that during this period there is a marked rise in the concentration of arsenic in the plasma and displacement from the red blood cells.

The extra elimination of arsenic occurring as the result of a single injection of BAL is not sufficient to account for lessening of the toxic effects and the saving of life. It must be presumed that the arsenic retained in the

body after the administration of BAL is no longer capable of exerting its toxic action. Experiments in which the distribution of arsenic in the tissues of guinea pigs and rats was determined before and after the giving of BAL demonstrate that this substance is displaced from most of the tissues but that increased amounts are stored in the liver and spleen. This, with other evidence, suggests that in the process of forming the insoluble thioarsenite complex the arsenic is displaced from the tissues, whence it is carried by the blood stream, especially to the spleen and liver for storage in a nontoxic form. This distribution corresponds to that demonstrated for various kinds of particulate matter that is taken up by organs relatively rightly supplied by the reticuloendothelial system.

THERAPEUTIC APPLICATION

The results obtained in the treatment of certain toxic reactions in man occurring in the course of arsenical therapy have borne out the promise of the laboratory experiments. To date several hundred cases of poisoning from various arsenic compounds have been treated in this country and in England, and a favorable response is believed to have been obtained in at least half of these. The best results have occurred in patients with arsenical dermatitis and encephalopathy, but there are also indications of the value of BAL in agranulocytosis and other blood dyscrasias. The results in hepatitis occurring in association with arsenical therapy have thus far been disappointing. The evaluation of BAL therapy has been complicated by the seriousness of the conditions treated and the consequent necessity for concurrent supportive therapy and supplementary medication. A much longer experience is necessary before final conclusions can be drawn, but there appears to be little doubt that in BAL there is available for the first time a specific agent for overcoming the toxic actions of arsenic.

Following the impressive results obtained in the treatment of experimental lewisite and Mapharsen poisoning, the value of BAL in poisoning by the mercuric salts has been investigated. Experimentally it was shown at the Edgewood Laboratories to be more effective than any other therapeutic procedure available in saving animals from otherwise fatal doses of mercury. This treatment is unique in overcoming the toxic effects, even when administered a considerable length of time after the mercury. It is extremely difficult to evaluate the efficacy of BAL in accidental and suicidal mercury poisoning in man, because of the uncertain and variable quantities of mercury taken and the impossibility of obtaining a comparable control series. However, it appears significant that in the series reported by Longcope, all of 30 consecutive cases treated with BAL survived, including some apparently seriously poisoned patients whose recovery was not otherwise to be expected.

BAL has also been shown to be of definite value in the treatment of experi-

mental cadmium poisoning, but relatively large doses are required. Furthermore, animals protected from the early toxic effects of cadmium may die later. BAL appears to be of no value in acute lead poisoning, and it has been reported as ineffective in neutralizing the toxicity of silver salts in the eye and in the removal of silver deposits in argyria in rats.

In clinical use, BAL is given by intramuscular injection in oil. The preparation generally employed is the one developed by Eagle, a 10 per cent solution in peanut oil containing 20 per cent benzyl benzoate to increase its solubility. The recommended dosage is 2.5 mg./kg., repeated four times at intervals of four hours during the first day and once or twice daily during the subsequent six days. With this dosage less than 1 per cent of the injections have caused minor toxic symptoms. In the treatment of poisoning by the arsenical war gases, larger amounts are recommended; namely, 4 mg./kg. in each dose.

The Action of Related Dithiols

A large series of dithiols has been synthesized. Many of these have been tested against lewisite and other arsenicals and their pharmacologic actions studied. In many instances slight changes in chemical structure have resulted in marked changes in the character of the effects produced on living tissues. These studies form a chapter of great pharmacologic interest, illustrative examples of which will now be given.

The ethyl ether of BAL is an extremely effective agent against poisoning by the arsenicals. In cats this compound is more effective than BAL in preventing the toxic effects of Mapharsen, and it is also of value in the treatment of poisoning by cadmium. BAL is of little if any benefit in the treatment of poisoning by arsine, whereas its ethyl ether exerts a strong protective action in the monkey, rabbit, dog, and cat. It was first shown by Kensler and Rhoads that not only is the survival time increased or death prevented, but the intravascular hemolysis is delayed and decreased and renal damage is prevented. The relatively high degree of protection afforded by the ethyl ether of BAL has also been demonstrated in vitro where the inhibition of the oxygen consumption of isolated tissue by arsine is prevented. The mechanism of this protection has not been elucidated. Arsine does not react directly with the dithiols in aqueous media.

Administered in large but sublethal doses to cats, the ethyl ether of BAL causes an irreversible injury to the cells of the central nervous system, resulting in abnormal posture and behavior patterns. It is also of interest that the vascular contraction described for BAL is shared by its ethyl ether, but in the case of the latter the effect is not readily reversible. It is possible that some of these differences are due to the relatively high fat solubility of the ethyl ether.

Several acetamido-methyl ethers and an acetate of BAL when injected into cats cause a marked pericardial effusion not seen with the other dithiols studied. The mechanism of this phenomenon requires further study.

Thus, not only do the dithiols have important practical applications, but they have contributed much information to pharmacologic theory, and it can be confidently predicted that they will serve as valuable tools in future biochemical studies.

DI-ISOPROPYL FLUOROPHOSPHATE (DFP)

Interest in the fluorophosphates was stimulated in World War II by information obtained from war prisoners indicating that Germany had prepared quantities of an alkyl derivative of fluorophosphoric acid for possible use as a war gas. It was probably the "nerve poison" about which rumors were current at the time. Earlier di-methyl and di-ethyl fluorophosphate had been prepared in Germany by Lange and Von Krueger, and the literature on the fluorophosphates was reviewed in Barth's *The Chemistry of Fluorine,* published by Springer during the war.

Investigation of the fluorophosphates was initiated in England. The chemical work was done under the direction of McCombie and Saunders and the biologic testing under Adrian. A series of alkyl derivatives was prepared and their toxicity to various species of animals was studied. A striking feature of their action was an intense, long-lasting constriction of the pupil, and this suggested an action similar to that of physostigmine on cholinesterase. Studies by Mackworth in Dixon's laboratory showed this to be the case. Not only was the cholinesterase more sensitive to di-isopropyl fluorophosphate (DFP) than to physostigmine, but the action was much more prolonged; indeed, it was impossible to obtain any evidence of reversal by the methods effective for physostigmine.

Following the British work, detailed studies on the kinetics of the reaction were carried out in the Biochemical Division at Edgewood. Inhibition of cholinesterase by DFP is almost immediate and is not reversible by any known means. The esterases of various tissues show wide differences in sensitivity to DFP when studied in vitro and corresponding inhibition of activity in the same tissues when the drug is administered systemically.

The only definite pharmacologic effects of DFP are those related to its inhibitory action on cholinesterase. In the absence of this enzyme acetylcholine accumulates at certain neuromuscular junctions and at nerve synapses; that is, at nerve endings in skeletal muscle and at the endings of autonomic nerves having a cholinergic function. The resulting effects are similar to those obtained by electrical stimulation of the corresponding nerves or the injection of acetylcholine. The so-called muscarinic effects of DFP (stimulation of organs having a cholinergic innervation) include

salivation, lacrimation, sweating, miosis, spasm of ciliary muscles, bronchial constriction, increased gastrointestinal activity, a fall in blood pressure, and slowing of the pulse. The chief so-called nicotinic effects are, first, stimulation of autonomic ganglia, resulting in additional effects attributable to the sympathetic nervous system, including pilomotor stimulation, dilatation of the pupil, and secretion of the adrenal glands; and second, stimulation of striated muscle at the site of the nerve endings, resulting in fibrillary contractions. As is the case with other drugs having a cholinergic action, large doses of DFP act on the central nervous system to produce hyperexcitability and convulsion. Thus the pharmacologic effects of DFP closely resemble those of physostigmine (eserine), whose actions are also believed to be mediated by its property of inhibiting the activity of cholinesterase. Unlike the DFP-esterase complex, however, the esterase regains its activity when the physostigmine is removed by dialysis or after its destruction in the body has come about.

In men exposed for brief periods to very low concentrations of DFP in the inspired air there occurs a remarkable reduction in the cholinesterase activity of the blood plasma. In many cases determinations of serum cholinesterase activity showed a decrease to 1 to 5 per cent of the pre-exposure level. Restoration to normal activity was a gradual process requiring from two to three weeks. It is noteworthy that in spite of this profound effect on the cholinesterase signs and symptoms were minimal, consisting in a few of the cases of mild effects during the first twenty-four hours, attributable to cholinergic stimulation. Similar changes occur in normal subjects following the intramuscular injection of 1 to 3 mg. of DFP.

The cholinesterase of the tissues is influenced to a much lesser extent. Thus, the activity in the red blood cells in the subjects described above was reduced to 70 to 80 per cent of its normal value. The problem has been investigated in more detail in animals, in which it is possible to determine not only the extent of esterase inhibition in different tissues, but also the rate of regeneration by measuring the activity at various intervals of time after giving DFP. The lethal dose of DFP varies rather widely in different species and is apparently related to the susceptibility of the brain esterase; the animal dies when the brain esterase activity approaches zero. However, a very marked reduction is compatible with survival. It was found in the Edgewood Laboratories, for example, that a group of rabbits receiving 0.3 mg./kg. by vein, which is approximately an LD_{10} dose, had one hour later a brain esterase activity 16 per cent of normal. The serum cholinesterase activity returned to normal in five days and that of the red cells in ten days, whereas it required from one to two months for the brain to regain its normal activity. The slow rate of recovery is strong evidence in favor of the view of an irreversible inactivation of cholinesterase by DFP and the regeneration of new enzyme.

It has been observed that cats recovering from a single large dose of DFP frequently show long-persisting changes, which may represent permanent neuromuscular damage. Weakness and loss of muscle control resulting in an ataxic gait are characteristic. Dogs given frequent small doses of DFP over a period of months gradually developed difficulty in swallowing and were shown at autopsy to have a widely dilated esophagus, presumably due to spasm of the cardiac sphincter.

While the effects of DFP are long-lasting, the compound is eliminated with great rapidity from the blood and tissues. Thus, within a half-hour after the intravenous injection of a large dose, blood samples lose their power of depressing the activity of esterase preparations, which may be taken as proof of the absence of free DFP. Other experiments have shown that rabbit and human serum, red cells, and tissues, especially those of the liver, contain an enzyme that accelerates decomposition of fluorophosphates to the alkyl phosphoric acid, hydrogen, and fluoride ions.

An interesting and at first sight paradoxical pharmacologic phenomenon is seen in the relation between the actions of physostigmine and DFP injected successively in cats. A small dose of physostigmine protects against a subsequent large dose of DFP. The symptoms are greatly reduced and the animals survive doses up to fifty times the usual fatal one. When the order of injection is reversed, the animals exhibit an increased and long-lasting sensitivity to physostigmine. The latter phenomenon receives an adequate explanation on the basis of the property possessed by both drugs of reducing cholinesterase activity. Thus, when the reserves are depleted by DFP only a fraction of the usual fatal dose of physostigmine is necessary to reduce the activity to a level incompatible with survival.

That the same phenomenon does not hold when the drugs are given in the reverse order is a problem of great pharmacologic interest. The reason for the high order of protection against DFP poisoning afforded by the prior injection of physostigmine has been elucidated by in vitro studies at Edgewood, in which it was demonstrated that this drug completely protects the esterase against inactivation by DFP. The same thing can be shown in the intact animal, where it has been demonstrated that the fraction of esterase inactivated by physostigmine is not destroyed by a subsequent dose of DFP, since recovery occurs in the course of a few hours as the physostigmine is eliminated. In test-tube experiments, it has also been shown that acetylcholine prevents the destructive action of DFP on cholinesterase. On the other hand, it affords little or no protection in the intact animal. This is probably because acetylcholine is quickly destroyed in the blood stream.

The properties of DFP, especially its long-lasting action, suggest its possible value in the treatment of conditions in which neostigmine is useful, such as myasthenia gravis and glaucoma. The possible therapeutic applications of this substance are now under active investigation. Preliminary results in myas-

thenia gravis indicate that DFP relieves the muscle weakness for longer periods than does neostigmine, but that the extent of improvement in muscular force is much less. This is true even though the reduction of the cholinesterase activity of the serum and red blood cells is far greater in the case of DFP. When it was given intra-arterially to patients with myasthenia, a striking localized increase in muscle strength occurred and lasted from eight to ten days. The place of DFP in the therapy of myasthenia gravis remains to be decided.

In the treatment of increased intraocular pressure by local instillation, DFP promises to be superior to other parasympathomimetic drugs. Not only is it effective when other drugs have failed, but fewer instillations are necessary, one application every five to ten days being sufficient in many patients.

As a tool in pharmacologic and physiological research, DFP promises to be of the greatest value. For the first time a means is provided for the complete elimination of cholinesterase in tissues. Already it has been shown in preparations treated with DFP that neostigmine is effective in stimulating skeletal muscle by direct action, whereas it had always been supposed that its effects were mediated through depression of cholinesterase activity. DFP should provide decisive evidence on the role of acetylcholine in nerve conduction, a field of investigation that is now occupying the attention of several laboratories.
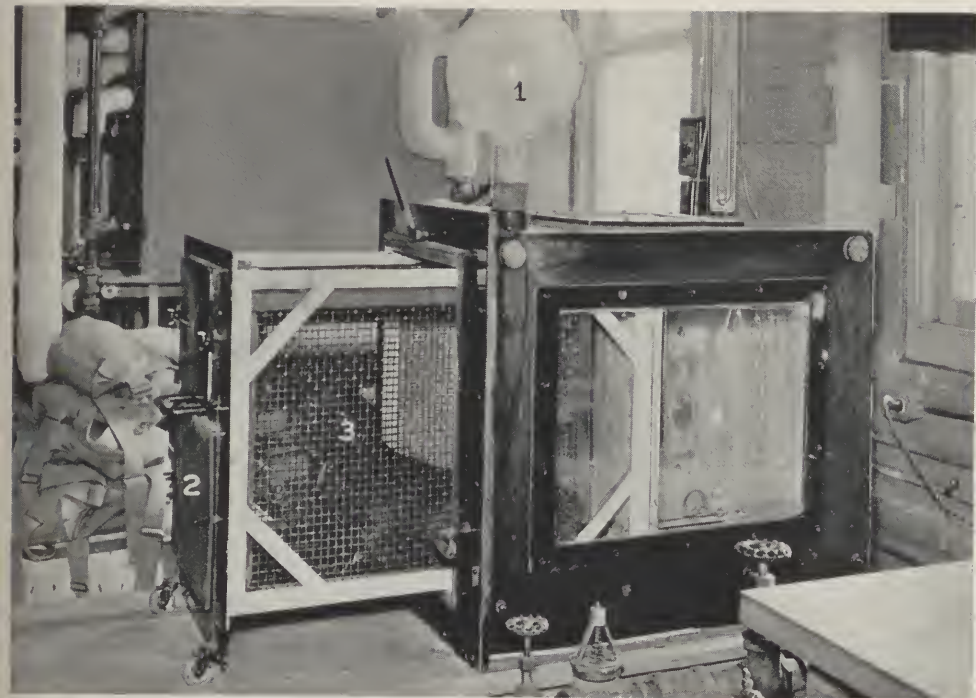
FIGURE 66. *A typical dynamic chamber.*

FIGURE 68. *Photograph of lungs of a dog sacrificed 1 to 2 days after exposure to phosgene.*
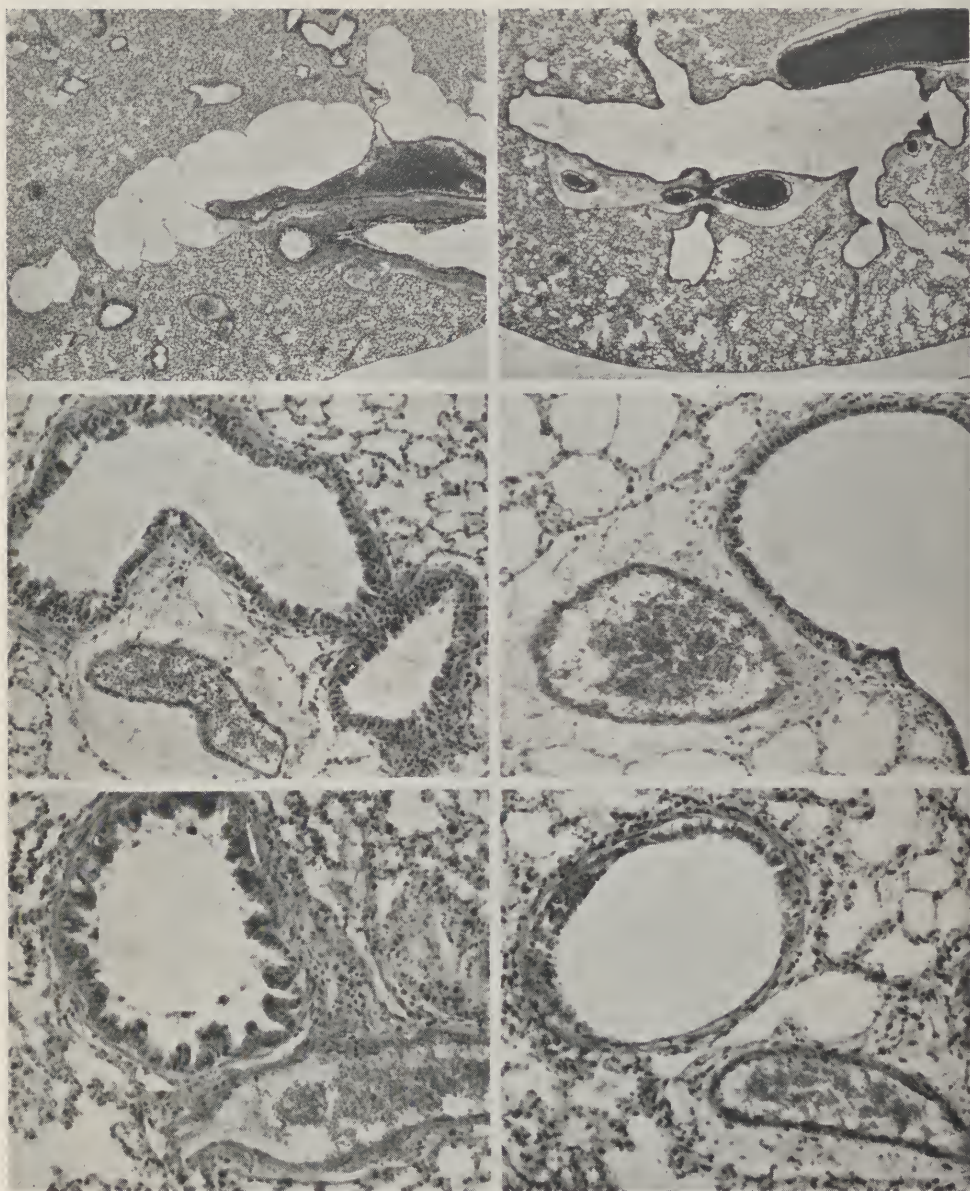
FIGURE 70. *Sections of mouse lung after various exposures to phosgene, with or without HMT prophylaxis.*

FIGURE 71. *Radiograms of chest after accidental exposure to phosgene.*
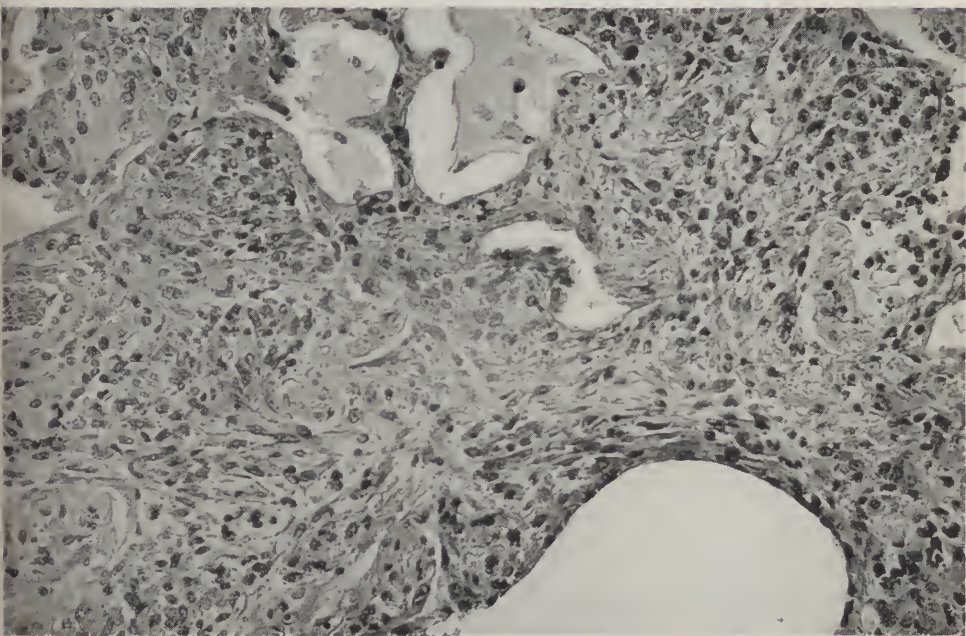
FIGURE 72. *Bronchiolar organization with extension of the process into the surrounding alveoli in a dog that died 72 hours after exposure to phosgene.*
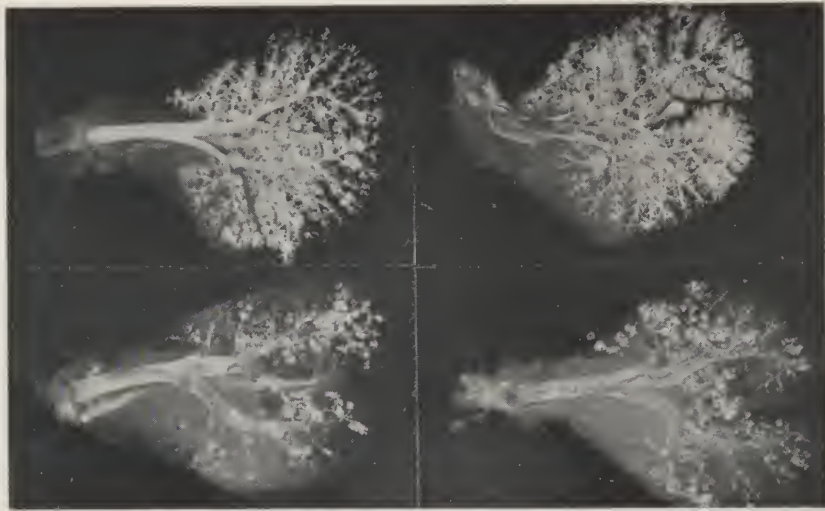
FIGURE 75. Radiograms of rats (A) and rat lungs (B) obtained by pouring a thin suspension of barium sulfate down the trachea immediately after sacrificing the animal.

CHAPTER XXXVII

RECENT RESEARCH ON RESPIRATORY IRRITANTS

RALPH W. GERARD

O N APRIL 22, 1915, gas warfare was born when the Germans released a cloud of chlorine over Allied troops at Ypres. The surprise attack completely ruptured the Allied lines and led to some 5000 battle casualties. During the remainder of World War I American troops suffered 70,000 gas casualties, as compared with somewhat over twice that number due to gunshot. Two per cent of the gas casualties and 8 per cent of the explosive ones died. Although most of the gassed soldiers recovered, and with essentially no residual damage, the immediate effect was so dramatic and disturbing that a mass abhorrence for gas warfare was generated. The following quotation from an article by G. W. Norris states well the impression of an expert clinician and summarizes also the therapeutic status at that time.

A field hospital full of freshly and badly gassed men is, in the estimation of all who have had an opportunity of seeing it, the most horrible and ghastly sight of the war. Even the man who has received multiple and severe wounds, when he has been splinted, put to bed, and given his morphine, is relatively comfortable; but to see a hundred or more men, hale and hearty a few hours before, slowly strangling to death from pulmonary edema with gradually increasing dyspnea, cyanosis, and pallor, making futile efforts to expectorate and to assist their breathing by voluntary effort and muscular contortions, until exhausted they pass from semidelirium into stupor, collapse, and death, is a never-to-be-forgotten sight, a sight which makes one clench one's teeth and curse the Hun who started this dastardly infamy. This is phosgene! But can nothing be done? Yes! The cyanotic cases are promptly bled, one pint, sometimes two. The ward looks like a shambles because in hurrying from bed to bed, twenty to thirty in a row, the spurting blood has left its trace upon bed and floor and linen. Meanwhile oxygen is being administered to greedy mouths while hands are loath to loose the bag when their five minutes of respite are over. For never are there enough bags for all, and the precious gas we must not waste, for it has been no small task to bring these great iron tanks up to the front. Opium we dare not use, for it checks an oft life-saving cough. But the gray cases, what of them? Lying about with a clammy skin, too weak to move or even care. Some venturous spirits say that one should bleed and then transfuse, but most that we should not meddle.[1]

[1] G. W. Norris, in *Transactions,* College of Physicians, 3rd Series, 41:120 (1919).

In World War I we learned about gas warfare from clinical experience. No laboratory information was available, at least to the Allies, at the time when they were faced with the urgent need of devising some sort of treatment for gas casualties. Improvisations and emergency actions were inevitable, and even when laboratory investigation was initiated activities were still prosecuted in an atmosphere of hurry and pressure. With the war's termination, interest in this field lapsed; almost no investigation was continued in this country and but little in England.

In World War II the situation was almost exactly reversed. The use of gas was anticipated from the start, and many research teams were set up to explore its action and the treatment of gas casualties. Although research was prosecuted under pressure, there was not the burning need to find some immediate treatment for gassed comrades, and much careful and thorough research was possible. But gas was not used, and the experimental findings of the present have not been subject to clinical check except in a very few isolated accident cases.

It is hardly surprising, then, that much of the theory and practice of World War I has been disproved or supplanted by the recent work. Until still further clinical experience is available, however, it would be unwise to follow uncritically the results in animals. This is the truer since the human experience was based largely on doses of gas that on the average led to a mortality of only about 2 per cent of the exposed persons, whereas animal experiments have dealt with doses killing nearer 50 per cent or even higher proportions of the exposed animals. Such doses are almost necessary in experimental work, since otherwise prohibitive numbers of animals would be required to test the influence of variables under study. Even with the experimental mortality levels used, as many as 3000 mice were needed in a single series.

It is obvious from what has been said that the immediate practical results from the current studies have been few; indeed, in the area of the pulmonary irritants, of which phosgene will be taken as a typical example, no effective treatment has emerged. True, several prophylactics have proved effective, and one, hexamethylene tetramine, extremely so; but even this substance was known before the beginning of World War II and had been explored by the Russians during World War I. This is not to say that the recent work was valueless. It has given us far deeper understanding of the mechanism and action of these agents, both chemical and physiological; it has cleared away much misunderstanding; and it has pointed the directions in which further research may be expected to yield profit. Further, and independently of gas warfare, a great deal of valuable knowledge of respiratory and circulatory mechanisms has been accumulated, much of which will certainly be applicable to civilian biology and medicine. It has been said, in connection with the development of the atomic bomb, that physics had for twenty years been ripening the apples on the tree of knowledge, and that the engineering de-

velopments that led to the actual explosive merely shook down the apples. In this field, we may safely say that the war work has led to further ripening and that sooner or later the apples will be shaken down.

PAST AND PRESENT STATUS OF AGENTS

The various lung-irritant gases that proved effective during World War I — phosgene, chlorine, diphosgene, chlorpicrin, and cyanogen chloride — and even the vesicants, like mustard, contain chlorine or other halogen atoms in the molecule. By hydrolysis, then, the strong hydrochloric or similar acid would be liberated in the tissues and was supposed to be the active agent producing damage. To be sure, hydrochloric acid itself was known to be far less toxic, but this was explained by the greater penetration into the cells by the organic halogen compounds, which, hydrolyzing within instead of outside of them, could be much more damaging. This fitted also with the clinical finding that a relatively long period, usually many hours, elapsed between the inhalation of the gas and the appearance of overt symptoms, this latent period being related to the time required for the intermediate chemical reactions. The symptoms that followed the latent period were those of severe pulmonary damage, with edema, and still later those of severe circulatory disturbances, often ending in a shocklike state and death. The specified treatment during the latent period was absolute rest, despite the serious handling problem that resulted when considerable numbers of casualties were encountered in the line. During the stage of developing lung damage and cyanosis, bleeding was recommended, followed still later by transfusion. The use of morphine or other sedatives was proscribed, and oxygen inhalation was strongly favored.

The present status is strikingly different. New agents have been developed, and for these, as well as for the more familiar ones, it has been demonstrated that liberation of acid plays at the most a minor role in their toxicity. Rather, the active halogen serves as a point for attaching the remainder of the molecule to critical tissue components. These are thus inactivated, and in their absence cellular damage ensues. The changes start at once; there is no true latent period. In the lungs edema formation, even with the usual doses, has been shown to commence within minutes, and after extreme ones it begins almost instantaneously. Nonetheless, the absolute rest insisted on earlier has been shown, on the basis of animal experiments, not to be essential. Much greater leeway is thus possible in the handling of early gas cases. The use of morphine is now also allowed in cases needing quieting, since it has not been shown to be harmful in the recent experiments. Conversely, the former extensive bleeding and transfusion maneuvers have come to be considered at best worthless and sometimes harmful and are no longer accepted. Even in the case of oxygen inhalation, which certainly gives symptomatic

relief and prolongs survival in severe cases of gas poisoning, it is extremely doubtful whether this procedure, although still unquestionably a desirable maneuver, brings about a reduction in mortality. It may be that oxygen with positive-pressure exhalation has some real value in decreasing mortality, but this has not been shown in animal experiments. The remainder of this chapter will be devoted to a brief summary of the evidence that has led to such a drastic change in views.

EXPERIMENTAL EVIDENCE[2]

Many lung-irritant gases or aerosols are now known. Those inherited from World War I — phosgene, diphosgene (superpalite), chlorpicrin, chlorine, and cyanogen chloride — have little direct action outside the respiratory tract, except for some eye injury by the more irritating ones, especially chlorine. Irritation by chlorine, and less by chlorpicrin, is also manifest in the upper-respiratory passages to a far greater extent than is irritation by phosgene. This may lead to immediate coughing and apnea, to gagging and vomiting, to reflex bronchoconstriction and laryngeal changes with stridulent breathing, and, with sufficient doses, to necrosis of the respiratory mucosa and death. Irritation with phosgene is minimal. No conjunctivitis follows the usual exposure, and no abrupt respiratory alteration or damage to the upper mucosa is seen. An early reflex slowing of the heart is one piece of evidence, however, that even in this case some stimulation of the respiratory tract ensues.

Cyanogen chloride is also a lung irritant, with an action much like that of phosgene, but in addition it acts, like cyanide, as a systemic poison. Mustard and lewisite produce severe lung damage when inhaled, but they act mainly as vesicants and less as systemic poisons. Cadmium fume, not infrequently inhaled as a result of industrial accidents, is a severe lung irritant as well as a systemic poison. It causes almost no initial irritation but leads, over a period of days, to lung edema and hyperplasia. Still other lung irritants have developed during World War II. So far as the irritant action of all these agents is concerned, phosgene can be discussed as the typical one.

PHOSGENE

Dosage

The technics of exposing animals to gases at desired concentrations and under regulated conditions have been enormously improved over such primitive procedures as allowing a certain amount of the agent to evaporate

---

[2] The great bulk of the work here referred to was carried out by four research teams operating under the Committee for the Treatment of Gas Casualties at Northwestern and Yale Universities and the Universities of Chicago and Pennsylvania. Full references are given in the Bibliography.

into a closed chamber. Various devices are now in wide use for vaporizing
the agent or otherwise mixing it with air, at the desired temperature, hu-
midity, concentration, and the like, and for passing this mixture at a rapid
rate through so-called "dynamic chambers," so that concentration is main-
tained essentially uniform in time and space (Fig. 66). Analytical methods
for these substances are now far more sensitive and precise. Furthermore, it
has been possible to follow the rate of hydrolysis, the influence of breathing
and air exchange, and the percentage of retention in the course of passage
through the respiratory system, and in additional ways to define with con-
siderable precision the absolute dosage given.

Under usual exposure conditions, with a group of mice, for example, sub-
jected to phosgene for a determined concentration (C) and time (t), an
LC_{50}[3] is obtained for some $C \times t$ dose. The dose that kills, say, 90 per
cent may be five- or tenfold the dose that kills only 10 per cent; that is, some
animals can survive an exposure ten times the one that kills others (Fig. 67).
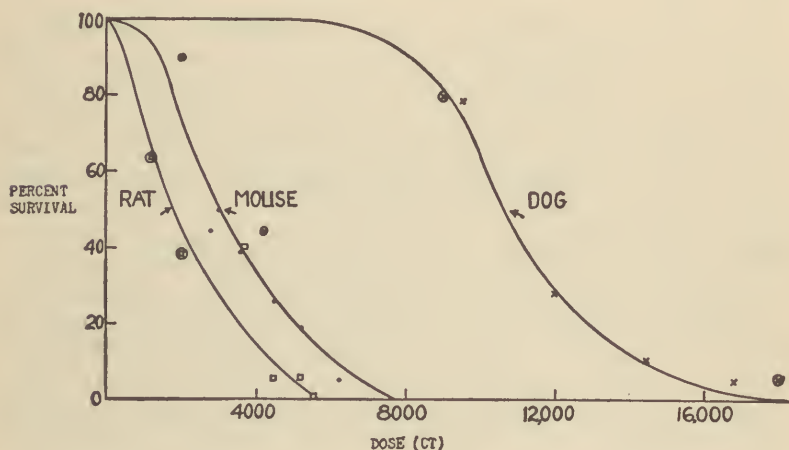
FIGURE 67. *Curves showing mortality against phosgene dosage
for three animal species.*

But with control of the many variables involved in absolute dosage, this
range can be greatly reduced. Thus, for a defined absolute dose of phosgene,
in a series of goat experiments essentially all the animals retaining less than
about 1 mg./kg. survived, and those retaining this amount or more died.
Within a given species, young animals are definitely more resistant than
older ones. Also, rather surprisingly, animals that have survived one ex-
posure, instead of becoming susceptible, are distinctly more resistant to sub-

[3] The concentration lethal for 50 per cent of animals treated.

sequent exposures. Further, there is of course a considerable species difference in susceptibility depending on differences in metabolic rate, lung size, ventilation, mediastinal thickness, and the like. This problem also has been explored and the contributing factors partly analyzed, and from comparative data reasonable figures can be extrapolated for man, even without direct human experimentation.

Finally, it has been clearly recognized that Haber's law, $Ct = k$, is not valid except for intermediate ranges of C and t. The effect of a very brief exposure to a very high concentration is quite different from that of a longer exposure to a lower concentration; in fact, the physiological and pathologic results really constitute two different disease entities. With very high concentrations, for example, the lung tissues are "cooked." The blood in them is clotted at once, and animals die within a few moments with no lung edema but with the formation of large amounts of acid hematin.

Site of Action

One important clarification has come from the proof that phosgene acts immediately and strictly locally on the lung tissues. (Not all pulmonary irritants are so circumscribed in their action; for example, cyanogen chloride.) The agent as such does not get past the lungs and has no effect on the rest of the body. This would follow almost inevitably from the extremely rapid hydrolysis of phosgene in the presence of water, a fraction of a second sufficing to destroy most of it; but the evidence is even more direct than this. If a plug is placed in a main bronchus of a dog, the animal exposed to a dose far exceeding the normal lethal dose, and the plug then removed from the protected lung and placed in the other bronchus (to keep edema fluid from spilling over), the animal so treated and exposed will normally live indefinitely (Fig. 68). This is so despite extreme pathology in the exposed lung, the retention in the body of many times the lethal amount of phosgene, and the loss of large quantities of plasma fluid — a point of importance later.

Another line of evidence, leading to a similar conclusion, is obtained by crossing the circulation between two dogs. If one of these is gassed and the other not, and the two are separated after some hours of cross-circulation, the exposed animal dies, but the other survives essentially undamaged. Another experiment which is of similar import is the following. Large amounts of blood from a heavily gassed animal can be transfused into another one, the recipient showing no ill effects. Finally, doses of phosgene or diphosgene far above those that are lethal on inhalation can be administered intravenously or intraperitoneally with, at the most, some local damage. It follows that the action of phosgene is exerted directly on the lung and that the damage to the organism is secondary. That the lung damage is prompt and severe has been amply demonstrated by histologic and chemical studies.

Pathology

Lungs removed from rats immediately after exposure fail to collapse as do normal ones; indeed, in all species a marked emphysema is probably the first pathologic finding. Within minutes the water content and the chloride content of the lungs begin to rise, and they continue to do so for hours. The blood content of the lungs rises during the first hours, but then falls off. The total lung weight increases steadily as edema fluid accumulates (Fig. 69).
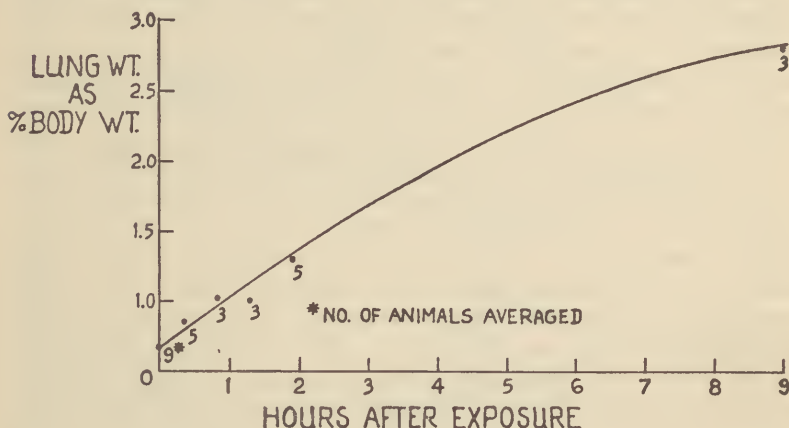
LUNG WEIGHT INCREASE AFTER DIPHOSGENE (RATS)

FIGURE 69. *Curve showing the prompt and continued increase in lung weight, due to accumulation of edema fluid, after exposure to a moderately strong dose of a lung-irritant gas.*

With very heavy doses, the lung becomes uniformly edematous and in five or ten minutes may become fully consolidated and resemble a piece of liver; but with ordinary doses these developments are slower and less uniform, so that the well-developed lesion shows patches of emphysema and of edema and congestion.

The development of histologic changes with time has also been carefully followed, especially with the aid of intravenous trypan blue, the passage of which into the lung tissues can be easily followed. Edema and staining are seen first in the tissues and blood vessels surrounding the bronchioles, then in the actual vessel walls, and only considerably later in the alveoli themselves. In fact, histologic damage at all stages, at least until pneumonia supervenes or late fibrosis occurs, is more striking in the bronchial tree than in the terminal air sacs (Fig. 70). Whether this means that, because of progressive hydrolysis in the moist air passages and sudden dilution in the

alveolar air, the agent actually does act less severely in the alveoli, or whether, as is more probable, the visible evidence of damage in the alveoli is less striking because of their simpler structure, is not determined. It is quite probable, however, that even the early peribronchial edema is really the result of a damming back of the lymph channels of the bronchiolar structures due to excessive amounts of fluid leaving the blood in the alveolar region. In any event, the edema fluid soon floods out from the alveoli and along the lumens of bronchioles and bronchi, unless these have become completely obstructed by swollen mucosa, mucous plugs, spasm, or other types of occlusion. The waterlogged lung comes to have four or five times its normal weight, a frothy edema fluid enters the larger air vessels and may even pour from the animal's nose and mouth, and, despite the patches of air remaining in the lungs, death follows severe interference with blood oxygenation.

When the outcome is more fortunate, and unless pneumonic complications result, resolution is almost as rapid as development, and in a few days the lungs return to a reasonably normal appearance (Fig. 71). Even dogs exposed to doses that kill over half the animals commonly show little residual disease a few weeks after exposure; but a small number of these, and a progressively larger fraction of animals receiving still larger doses (especially when kept alive by oxygen inhalation or by other maneuvers), show significant late changes. The bronchiolar epithelium proliferates and an obliterative bronchiolitis develops (Fig. 72). This may lead to a late asphyxial death just as surely as does the early lung edema. It is probably because of this proliferative late lesion that animals kept alive for weeks with oxygen may still eventually die and that mortality figures are not more improved by oxygen therapy.

Chemistry

We must turn back, however, from this picture of developed lung damage to examine more closely the chemical and physiological consequences of phosgene inhalation. When phosgene reacts with water it forms two molecules of hydrochloric acid and one of carbon dioxide, according to the first of the following equations. If it reacts with some other organic compound, according to the second equation, the hydrochloric acid will still be formed but the $=CO$ group will remain attached to that compound and will not appear as carbon dioxide gas. Since water is always present and reacts rapidly with phosgene, only those substances that react even more rapidly can effectively compete for combination with the $=CO$ group. These properties make possible a very simple test for phosgene reactions. Phosgene, or better diphosgene, is added to water or to an aqueous solution of the substance to be tested in a closed manometric system. In water the theoretical amount of carbon dioxide is released and measured. In the presence of an active combiner, which can completely displace water from the reaction, no carbon dioxide would be liberated (Fig. 73).

$$
\begin{array}{l}
\text{(1)}\quad O{=}C\underset{\text{Cl}\ \ \text{H:OH}}{\overset{\text{Cl}\ \ \text{H:OH}}{\Big\langle}} \ + \ \ \longrightarrow \ O{=}C\underset{\text{OH}}{\overset{\text{OH}}{\Big\langle}} \ + \ 2\,\text{HCl}
\end{array}
$$

$$CO_2 + H_2O$$

$$
\text{(2)}\quad O{=}C\underset{\text{Cl}\ \ \ \text{HXR}}{\overset{\text{Cl}\ \ \ \text{HXR}}{\Big\langle}} \ + \ \ \longrightarrow \ O{=}C\underset{\text{XR}}{\overset{\text{XR}}{\Big\langle}} \ + \ 2\,\text{HCl}
$$
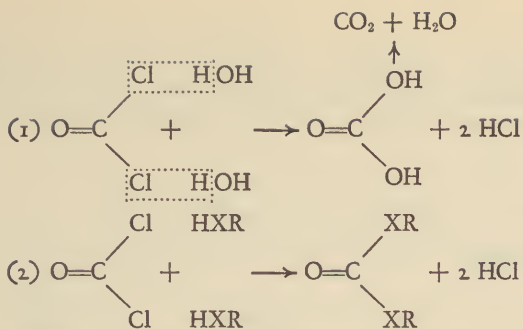
X = —NH—, —NHNH—, —NR—, —O—, —S—.
R = H, alkyl, aryl.
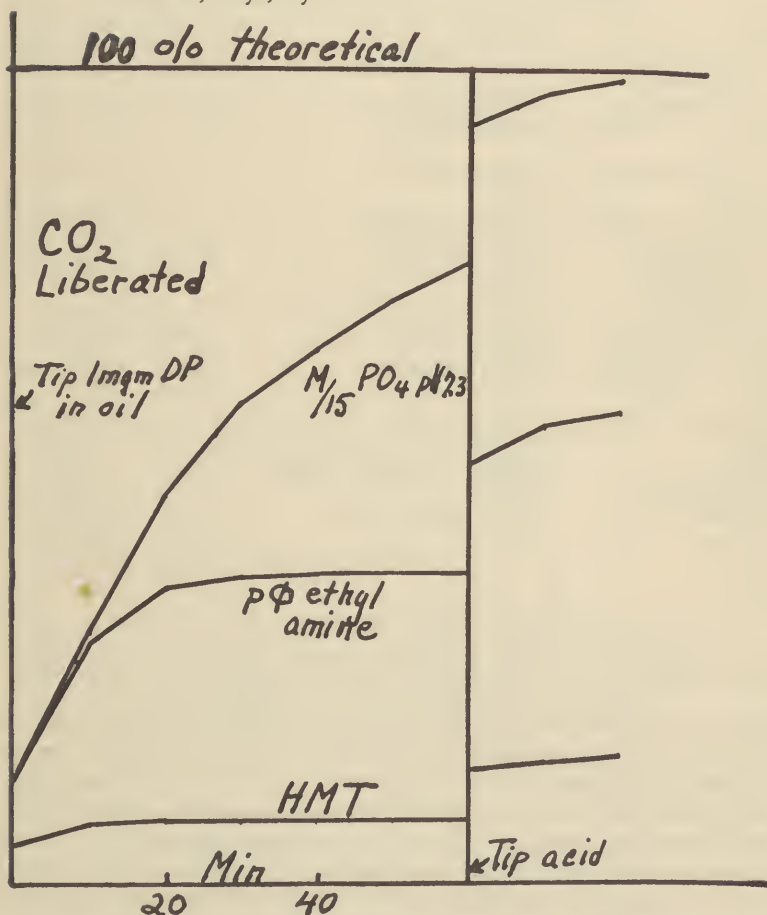
FIGURE 73. *Course of carbon dioxide liberation after adding diphosgene (in oil) to the aqueous medium.*

Actually, in nearly a hundred compounds tested such competition between the test substances and water gave results all the way from zero to over 95 per cent of the $=$CO bound (Table I). Highly reactive substances, when

TABLE I

Ability of Some Tested Substances to Bind the $=$CO Group of Phosgene.*

Name	Formula	% Diphosgene $=$CO Bound	Protection of Yeast
HMT	$C_6H_{12}N_4$	80+	Complete
1,2 diamino phenyl 4 sulfonic acid	$(NH_2)_2C_6H_3SO_3H$	80	Considerable
p-amino benzoic acid	$NH_2C_6H_4COOH$	70	Complete
Tri phenyl trimethylene triamine	$(C_6H_5)_3(CH_2)_3N_3$	70	Fair
Tri ethyl trimethylene triamine	$(C_2H_5)_3(CH_2)_3N_3$	55	None
Glycine ethyl ester HCl	$HClNH_2CH_2COOC_2H_5$	65	Considerable
Taurine	$NH_2CH_2CH_2SO_3H$	60	Fair
2 naphthylamine 6 sulfonic acid	$NH_2C_{10}H_6SO_3H$	50	Complete
Sulfanilic acid	$NH_2C_6H_4SO_3H$	45	Fair
b-phenyl ethyl amine	$C_6H_5CH_2CH_2NH_2$	35	None
Aniline 3,4 disulfonic acid	$NH_2C_6H_3(SO_3H)_2$	20	Complete
p-amino ethyl benzoate	$NH_2C_6H_4COOC_2H_5$	20	None
Urea	$(NH_2)_2CO$	0	None
Arginine	$HNCNH_2 \cdot NH(CH_2)_3CHNH_2COOH$	0	None
Sodium thiosulfate	$Na_2S_2O_3$	0	None
Methylene blue	$[(CH_3)_2NC_6H_3]=_2S \cdot N$	0	None
Coenzyme		0	None
Coagulin		20	Complete
Plasma		10	Fair

* Note that only active binders are effective in protecting yeast cells against phosgene; but some substances are of no prophylactic value even though they do bind phosgene.

not themselves toxic and when tolerated by the organism, might therefore be expected to act as prophylactics if present when phosgene is inhaled. By such tests hexamethylenetetramine did, in fact, prove about the most active phosgene-combiner and, as has been mentioned, it is by far the best prophylactic known for phosgene (Table II). Para-amino benzoic acid, another strong reactor, is able to protect yeast against phosgene action about as well as does hexamethylenetetramine, but for unknown reasons it is ineffective in mammals.

Table II

Tests of Some Prophylactic Agents Against Diphosgene (DP).*

DP mgm. × 10 min.	Agent	Animal	Dose (gms./K. Intraperit.)	Time Pre-Gassing (hours)	No. Animals	Per Cent Alive at (hours)							
						1	2	4	8	12	16	24	In-def.
.64 to .82 (nominal) .82	HMT	rats; mice	2 or less	0 to 6	113	100	96	84	71	60	52	46	42
	Control				83	91	82	68	35	7	5	5	3
	HMT	rats	2 +	¼	10	100	100	100	100	100	100	100	100
	Control				10	100	90	70	50	0	0	0	0
.66 to .79	Taurine	mice	2–6	½ to 1	55	100	100	94	84	73	63	47	31
	Control				60	100	98	93	65	40	25	10	5
.58 to .67	1,2 diamino phenyl 4 sulfonic acid	mice; rats	2–4	¼ to 6	106	100	100	98	93	84	77	51	29
	Control				60	100	100	98	75	64	45	31	13
.68	2 naphthylene 1 sulfonic acid	mice	0.8	½	20	100	95	85	65	50	40	25	12
	Control				19	100	90	65	30	20	15	5	0
.60 to .70	Coagulin	mice	atomized susp.	during 3 to 6 hours till gassing	30	100	100	100	97	87	57	33	20
	Control				30	100		100	93	73	27	7	0

*Note complete protection by hexamethylenetetramine in one series in which all unprotected rats were killed.

These two substances are examples of many nitrogen-containing chemicals, mainly primary amines (R—NH), that react vigorously with the phosgene molecule. (See equation 2 above.) Another group of compounds, containing the sulfhydril (R—SH) group, also react vigorously, although none of these has proved an effective prophylactic in vivo. Among such compounds is the dithiol BAL, which is extremely effective against various metallic poisons (arsenic, mercury, and cadmium) but is actually harmful in phosgene poisoning. Still another group of substances with which phosgene reacts, although rather less vigorously, are those containing hydroxyls (R—OH). Phosgene, then, on entering the lungs either reacts with water to form essentially harmless products or reacts with amino, sulfhydril, or possibly hydroxyl groups of the organic compounds of the tissues, or of compounds that have been especially added as prophylactic agents.

Phosgene has thus been shown to react with a number of the specific amino acids of tissues, with a number of the proteins containing these amino acids, with some hormones built of protein, such as insulin, and, most important of all, with various cell enzymes, all of which contain protein. A number of specific enzymes concerned with respiration, glycolysis, phosphate changes, and the like have been shown to be damaged in vitro by reasonably small doses of phosgene. As a consequence of such injury, the metabolism of cells in the walls of the lung vessels and other structures is interfered with. Direct measurements do show a small but definite decrease in oxygen consumption by lung tissue after exposure to the usual doses of phosgene. An interference with the glycolytic processes in yeast has similarly been demonstrated.

Another specific phosgene damage is that caused to the lung thromboplastin system. Thromboplastin, a lipoprotein that fosters coagulation, is richly present in the normal lung. After exposure to phosgene, the coagulating activity of lung extract is greatly diminished, and this may be of significance in relation to the increased permeability and edema produced by phosgene. In any event, thromboplastin is one of the few agents shown to have some prophylactic action.

It was stated above that the products of hydrolysis of phosgene are essentially harmless, although one of these is hydrochloric acid. The justification for this statement is as follows. Even for tissue preparations with the cells destroyed, or for relatively pure enzyme extracts, hydrochloric acid is far less injurious than is phosgene. Since impermeability to the acid is excluded in these cases, the action of phosgene must be due to something more than liberation of acid. Furthermore, the substance ketene acts much like phosgene and is even more toxic. It does not have halogen atoms in its molecule and so cannot form strong acid, but it does have a $=CO$ group that can combine with tissue substances in the same way that phosgene does.

Lung Physiology

Phosgene acts, then, in the lung and promptly combines with critical tissue substances, especially enzymes, and inactivates them. As a result, cell metabolism is interfered with, the liberation of energy is decreased, and the normal functioning of the cells, which includes the maintenance of their membrane properties, begins to fail. The most important physiological consequence of this is that the semipermeability of the cell membrane is lost. Normally, the proteins of plasma exert an osmotic pressure of some 25 mg. of mercury, tending to draw fluid into the blood stream. The hydrostatic pressure in the pulmonary capillaries is only about 9 mg., so that water in the alveoli is easily absorbed and plasma water does not leak into them. With semipermeability gone, the plasma proteins can also pass through the membrane, and their osmotic action is lost. Fluid, including the plasma proteins, can then be forced through the membranes of the lung capillaries, even by the slight pressure in the vessels, and edema results.

Many problems have been raised by the more careful study of this phenomenon in lungs. For example, when edema fluid begins to form it contains only a low protein concentration, indicating that semipermeability is at first not entirely lost. Later, the proteins appear in the same amount and proportions as in plasma; the globulins, which are large molecules, get through the membrane as easily as do the albumins, which are small protein molecules. Still, not all the membrane area has suddenly lost its impermeability after the usual conditions of exposure; for, under larger doses of phosgene or usual doses of another pulmonary irritant, the lung edema may be complete in a few minutes instead of a few hours. With usual doses, therefore, either small portions only of the total capillary membrane are damaged but become completely permeable, or the whole membrane is initially damaged only to a small extent. The former is probably more nearly the correct situation, with, however, some progressive membrane damage also occurring.

Conversely, after the acute injury by phosgene, although the edema takes days to resolve itself, it is highly probable that the capillary membrane has been restored to or toward normal much earlier than this. If protein-containing solutions are poured into the alveoli even of a perfectly normal lung, it takes over a day for any large amount of the protein so introduced to disappear from the alveolar content.

Phosgene produces little initial irritation. Nevertheless, as part of its damaging action it stimulates certain sense organs in the lungs and can bring about responses aside from the damage to capillary membrane. Nerve reflexes set up from sense organs in the respiratory tract, for example, bring about a marked slowing of the heart during the period of exposure (Fig. 74). Other reflexes may be involved in producing the early increase in the blood

content of the lungs. Still others may lead to constriction of the bronchioles, but this is still somewhat debatable.

Lung preparations isolated after exposure to phosgene or phosgenized in vitro do not show greater resistance than normal when fluid is perfused through the bronchial tree; nor has it been possible to demonstrate narrow-
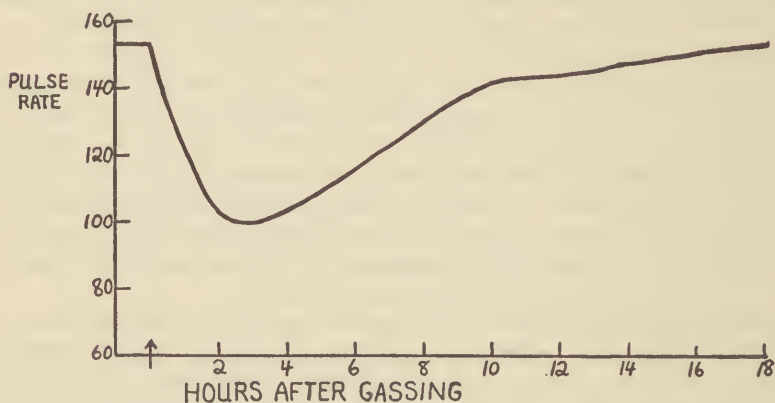
FIGURE 74. *Early slowing of heart rate in dogs after exposure to usual dose of phosgene.*

ing of the bronchi after phosgene, when these are made visible to x-rays by depositing on them an inhaled radio-opaque dust. On the other hand, some bronchial narrowing is indicated by the decreased collapsibility of gassed lungs, by greater changes in intrathoracic pressure with respiration (abolished by cutting both vagus nerves), by the failure of liquids poured into the trachea to penetrate as far into the bronchial tree as normally (Fig. 75), and the like.

Another possible action of phosgene is that exerted directly on the blood that chances to be in the lungs when the gas arrives. Actually blood cells in the phosgenized animal do show some tendency to form clumps or sludges, but this is not very striking, nor is there any significant amount of hemoglobin change, such as the formation of acid hematin, except after exposure to overwhelming doses of the gas.

A final possibility, worth mentioning here, is that phosgene liberates poisonous agents as a result of its action on the lung tissues. Histamine, for example, if liberated locally might spread within the lung and increase the capillary damage and permeability, or might be carried around the body and help to bring about the circulatory disturbances. Some histamine may, indeed, be formed, but the consensus of careful evidence is that this is completely unimportant. Certainly the addition of histamine does not exaggerate

phosgene symptoms, nor does the presence of an enzyme that tends to destroy it ameliorate them. Further, since neither the protected lung in a gassed dog nor the lungs of the unexposed dog of a cross-circulated pair show any damage, no considerable amount of circulating histamine can be present. These same arguments apply to another capillary-active substance, leukotaxin, which has been considered as a source of damage. Some such substance is richly present in the lung edema fluid after exposure to mustard or lewisite, but it is present only in negligible amounts, if at all, in the lungs or edema fluid from dogs gassed with phosgene or similar respiratory irritants.

Systemic Consequences

The picture thus emerging is reasonably clear. Phosgene acts promptly and vigorously to injure lung tissues, especially to destroy the semipermeability of the capillary membrane. The subsequent disturbances develop primarily from this effect; for the other possible actions — nerve reflexes, liberated chemicals, blood injury, and the like — are shown to be at most of secondary importance. Three disturbances to normal physiology might result from the outpouring of fluid into the lungs: interference with the gas exchange through the lungs, leading to hypoxia and hypercapnia; loss of blood plasma or plasma fluid, leading to circulatory inadequacy; and obstruction to the circulation through the waterlogged lungs, leading to back pressure on them and again to disturbed circulation.

The last of these loomed large in the minds of investigators during World War I. Clinically the right side of the heart was found to be enlarged, the neck veins were distended, and evidences of passive congestion were discovered at autopsy. It was to relieve an embarrassed right side of the heart that bleeding was recommended as an early therapeutic measure. Direct measurements of pressures in the pulmonary artery, right ventricle, and right auricle have now consistently shown, however, that there is no increased back pressure on the right side of the heart or the circulation. Actually these pressures fall progressively, in parallel with the fall in aortic pressure (Fig. 76). The few measurements of venous pressure that have recently been available in human phosgene cases have not shown a rise, and in the animal experiments when venous pressure has risen it has done so even with low pressure in the right side of the heart. There is therefore no true passive congestion; the occasional raised venous pressure is undoubtedly a result of interference with the normal aspirating action of the chest, due to altered respiratory movements and to the heavy, inelastic lungs. Further, other physiological experimentation has shown that the pulmonary circulation possesses an enormous reserve capacity. Removal of one lung in no way interferes with the pulmonary circuit or the right side of the heart. In fact, it is possible to constrict the common pulmonary artery by 70 per cent or more before any back pressure becomes manifest.
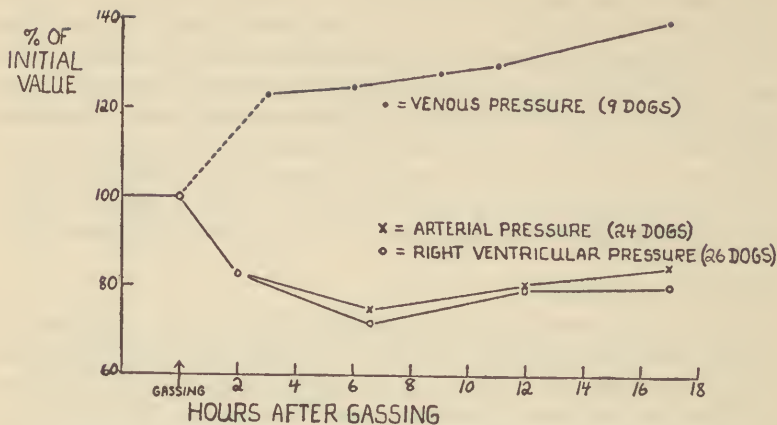
FIGURE 76. *Graph showing fall of blood pressure in the right side of the heart of the dog, in parallel with systemic arterial pressure, despite some rise in venous pressure.*
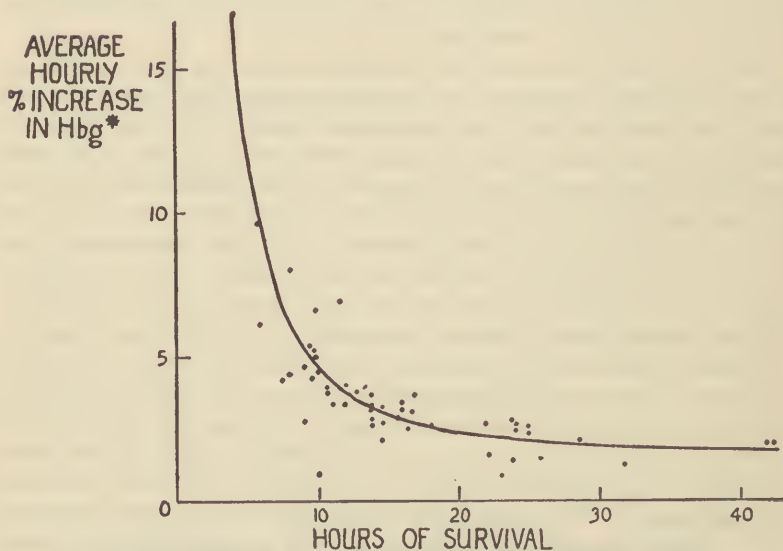
FIGURE 77. *Relation between rate of increase of hemoglobin percentage (rate of loss of blood fluid) and time of survival of dogs after gassing.* (After Bunting et al.)

The systemic consequences of phosgene poisoning thus reduce themselves to a combination of the first two factors, asphyxia and fluid loss, and both these in turn result from the translocation of blood fluid from the vessels into the spaces of the lungs. The evidence for prompt and severe flooding of the lungs has already been given. Actually, more water can be demonstrated in the damaged lung than can be accounted for as having left the blood — owing undoubtedly to some mobilization of fluid from the other tissues into the blood stream.

The loss of circulating fluid manifests itself in several ways. For one thing, as plasma leaks out the red corpuscles become more concentrated, as shown by a rise in the hematocrit and in hemoglobin concentration. Indeed, the rate of hemoconcentration in the early stages of phosgene poisoning has proved to be of considerable prognostic value; the more rapid the rise, the worse is the probable outcome (Fig. 77). (In some patients an initial slight hemodilution precedes concentration; it seems to improve the prognosis.) Along with such concentration goes an increased blood viscosity, which interferes with free circulation; and of course a decrease in total blood volume, directly measured by the dye method, also occurs. As a consequence of all this, the circulation time is prolonged, in many cases almost twofold (Fig. 78). The lowered arterial blood pressure and cardiac output contribute further to this outcome (Fig. 79).

To return to the lungs, the edematous organ is able to exchange both oxygen and carbon dioxide far less efficiently than normally. The oxygen content of venous blood becomes markedly reduced, and even in the arterial blood after it has passed through the lungs oxygen concentration and tension fall progressively further below normal. At the same time, carbon dioxide accumulates and, in the earlier stages of phosgene poisoning, there appears an acidosis of the respiratory type. (This is associated with an early and temporary fall in arterial oxygen, due mainly to reflex changes in the bronchioles, respiration, and circulation. The amount of fall has a prognostic value.) Later, with an inadequate supply of oxygen to the tissues, the carbon dioxide production also falls, and in the late stages a typical metabolic acidosis results from the entry into the blood of nonvolatile acid products of incomplete oxidation.

As a direct result of the lung damage and the outflow of fluid, then, the blood, containing less oxygen than normal, is circulated to the tissues in smaller amounts and at a slower rate than normal. To anoxic anoxia is added stagnant anoxia. If this process progresses, increasing tissue asphyxia results and the various body organs fail in their order of susceptibility. The nervous system loses its effective functioning relatively early, the heart relatively late, and in death from phosgene the heart continues to beat after respiration has failed.

It is not entirely academic, however, to raise the question of which of the
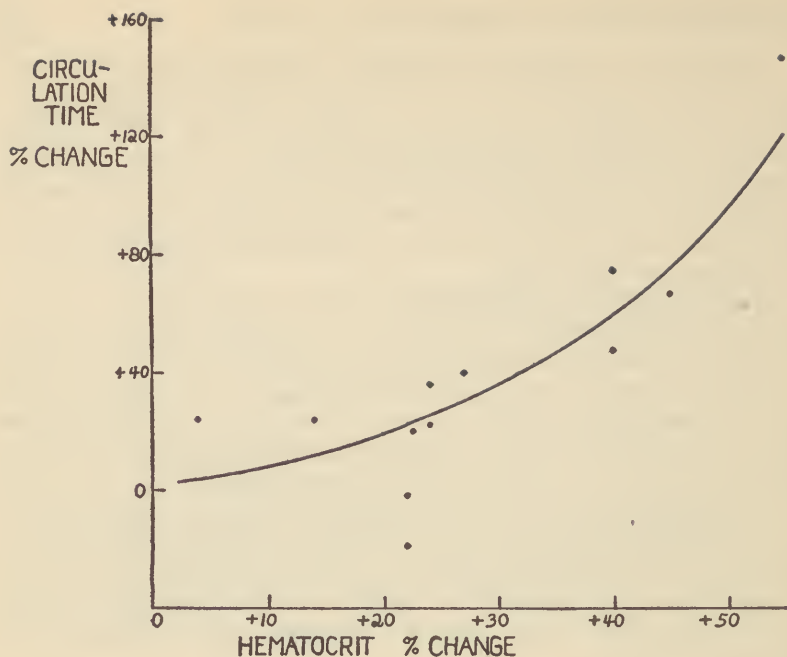
RELATION OF CIRCULATION TIME TO HEMOCONCENTRATION

FIGURE 78. *Relation between increased circulation time in dogs (slowed circulation) and increased loss of blood fluid (rise of hematocrit).*
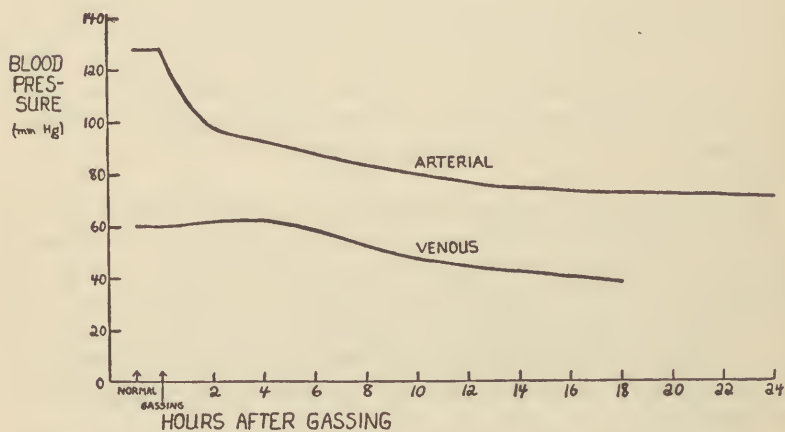
FIGURE 79. *Average course of arterial and venous blood pressures in the dog after exposure to phosgene.*

two factors is of predominant importance in death from phosgene. Extensive experiments have shown that animals die when their arterial oxygen content falls below a certain level, and not when the concentration of the blood rises to a certain level. Animals may survive a very high hematocrit reading and yet die later, when the blood is actually beginning to dilute again, but
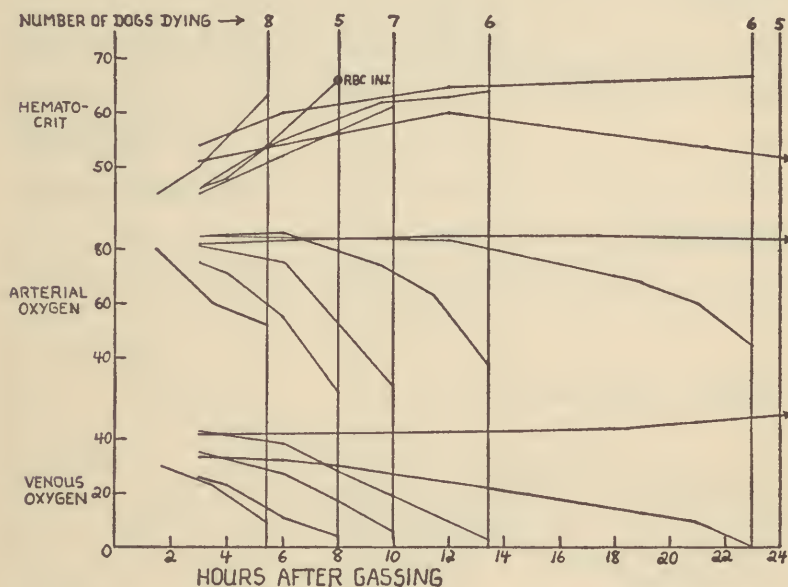
RELATION OF HEMOCONCENTRATION AND ANOXIA TO TIME OF DEATH

FIGURE 80. *Graph illustrating the nature of the fatal disturbance after phosgene poisoning.*

they never die unless the arterial oxygen content is progressively falling (Fig. 80). Rabbits fail to develop any significant blood concentration, but die in typical fashion. Dogs with one lung exposed and the other protected may show extreme hemoconcentration yet survive while the good lung continues to function and the blood oxygen remains elevated. In some such cases, however, after survival far beyond the usual time after a severe exposure, circulatory failure may indeed become critical, and the animal dies in a typical shocklike state. The same situation holds with animals given oxygen therapy (Fig. 81). The blood oxygenation may be kept up for some time and the animal may survive, at least into the delayed stages with bronchiolar hypertrophy, or in other cases until a progressive hemodynamic disturbance brings on shock. In all such late fatalities, a progressively widening

difference between arterial and venous oxygen content emphasizes the developing stagnant anoxia. Of the fatalities in untreated animals, less than 10 per cent could be attributed to circulatory failure, whereas of the fatalities
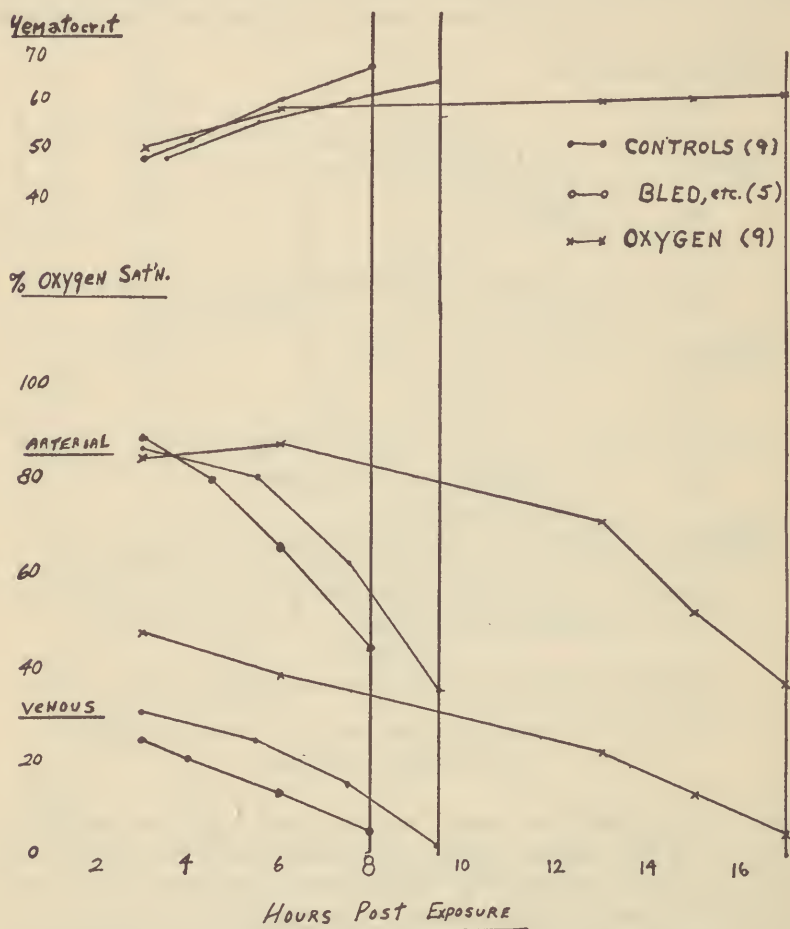
FIGURE 81. *Graph comparable to that in the preceding figure, showing the fall in blood oxygen before death.*

in animals kept alive beyond the usual time, with oxygen or a protected lung or in similar ways, over a third of the deaths were due to circulatory failure.

The restoration of tissue fluid by appropriate transfusion would theoret-

ically be of help in such cases, were it not for the fact that the leaking lung membrane will not retain added colloids. Infusion of saline particularly, but even of colloid solutions, including whole plasma or protein-rich concentrated plasma, thus does not lead to an improvement in circulating blood volume. Rather, the extra fluid placed in the vein merely raises the blood pressure and thereby brings about a further loss of fluid into the already edematous and flooded lungs. In animal experiments, infusion of any sort or bleeding of any amount, or any combination of the two maneuvers, at best did not adversely affect the mortality figures. In most cases such handling seemed definitely deleterious.

THE TREATMENT OF LUNG–IRRITANT CASUALTIES

On the basis of such experimental results the older therapeutic procedures and dicta are being discarded. Absolute rest in the early stages is not essential (Fig. 82), and conversely the use of morphine or other moderate sedation
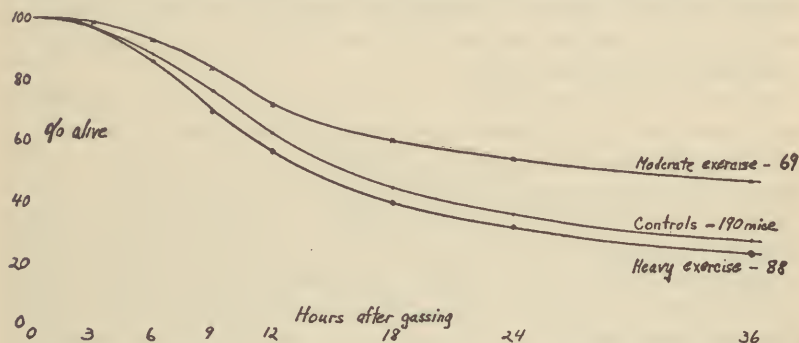
FIGURE 82. *Graph illustrating the effect of exercise on mortality after exposure to phosgene.*

in the later stages is not contraindicated. Bleeding at any time is unnecessary and often actually harmful, and transfusion of any material so far tried is also worthless or injurious. On the basis of the chemical and physiological clarifications resulting from recent research, every conceivable therapeutic maneuver has been explored in animal experiments. Unfortunately, none has proved to be really satisfactory. The ideal objective is of course to prevent the initial damage to the capillary cells. This can be done when an appropriate chemical is present to compete for phosgene at the time of its arrival. Not only are hexamethylenetetramine and related compounds thus effective, but also, on an unknown basis, magnesium carbonate dust or magnesium salt injection or some of the other alkaline earth compounds are moderately so.

Thromboplastin and the related concanavalin, derived from jack beans, have shown some prophylactic action, as have also the capillary permeability-decreasing K vitamin group of substances. Another agent, thymoxyethyl-dimethylamine, which antagonizes the action of histamine and also seems to hasten the hydrolysis of phosgene, has been strikingly effective in a few experiments in some species but completely disappointing in others. Animals dehydrated prior to exposure by deprivation of water and food also do rather better when phosgenized, and rats fed a carrot diet to increase their resistance to anoxia also do better. All such prophylactic measures are, however, impractical in terms of any real value. Even under war conditions, it will not do for troops to inhale magnesium carbonate or be perpetually dehydrated or to have their blood kept enriched with hexamethylenetetramine by daily administration. And, unfortunately, none of these maneuvers are of the slightest benefit when initiated even a few minutes, three or less, after exposure has occurred. (This, by the way, is further strong evidence for the immediate and local action of phosgene on the lung tissue.) Only one agent, posterior pituitary extract, has influenced mortality favorably when given after exposure, and even this has been accomplished only when it is also given before the gas is inhaled.

Spasmolytics have been given to relax obstructed bronchi, nerve-paralysant drugs administered to eliminate any possible reflexes, posturing or mechanical manipulation used to improve the drainage of lung edema fluid, quinacrine, quinine, and heparin injected to eliminate even the small blood clumping, emetine and other drugs given to constrict the pulmonary blood vessels, amyl nitrite inhaled and the spinal cord sectioned to reduce systemic blood pressure, colloidal and larger particles injected to close up the leaking capillary membrane, and calcium, adrenocortical extract, and vitamins C and P given to lessen capillary permeability. All these and many other maneuvers have been explored, but to no avail. Thus, it seems hopeless to prevent the membrane damage in the lungs or artificially to decrease the fluid passage across the damaged membrane. There is one possible exception to the latter statement. A maintained back pressure in the alveoli, such as can be obtained with positive-pressure respiration or expiration, could theoretically help. Recent clinical experience suggests that it does so in fact, although here again all animal experiments have failed signally to demonstrate any value in such a procedure.

Since most deaths result from interference with the gas exchange and are essentially anoxic, the one remaining hope is to combat the anoxia. If an animal can be tided over the acute edema and if hypertrophic lung changes can be avoided so that ultimate gas exchange through the lungs will be adequate, survival should be possible. Oxygen inhalation certainly works in this direction, but with advancing edema even this often fails. Some evidence suggests that oxygen at less than atmospheric pressure, mixed with helium

or with nothing (in a low pressure chamber), may be better than pure oxygen, because the lowered viscosity permits easier exchange through narrowed air passages.

Oxygen has also been administered extrapulmonically, by direct injection into blood vessels or the abdominal cavity, or, particularly, as large subcutaneous depots. In these cases also the amounts available are, under conditions so far explored, insufficient to be of great value to the animal. Theoretically, an animal could be kept alive by substituting the normal lung, even if completely damaged, or through some other means of aerating the blood. It has, in fact, proved possible to maintain one dog without any respiration by cross-circulation with another dog, but all efforts to make artificial lungs by some sort of mechanical contrivance have so far been defeated by technical difficulties. Other attempts to supply oxygen substitutes, such as intravenous methylene blue or hydrogen peroxide, have also failed to date. Nor would it be a very satisfactory solution to keep a phosgene-poisoned victim alive with an artificial lung or continued injections unless the damage to his own lung was sufficiently reversible and transient so that he could eventually regain its use. This involves solution of the problem of fibrosis following injury, a matter that has not yet received experimental attention.

CONCLUSION

Although the prospects for effective treatment of acute pulmonary irritant poisoning are not bright, the work devoted to this subject has not been useless. Not only has the problem been clarified and the mechanism of action of these agents largely exposed, but also much knowledge on the physiology of respiration and circulation has accrued. This knowledge will prove of real value in dealing with problems of industrial hygiene and accidental poisoning. Indeed, in the case of cadmium fumes, which are responsible for rather frequent industrial accidents and which act largely as a lung irritant and produce disease much like that caused by phosgene, an effective therapy has been developed, based on the studies on phosgene and on systemic poisoning with lewisite. Also, much of this knowledge will be directly applicable to other cases of lung edema or consolidation, as in pneumonia. Lastly, the great experience gained in the indications and conditions for using oxygen therapy is already paying high dividends in the civilian practice of medicine.

CHAPTER XXXVIII

PROTECTION AND TREATMENT OF THE SKIN EXPOSED TO BLISTER GASES

MARION B. SULZBERGER

THE STORY of the development of materials for the prevention and treatment of skin injuries caused by chemical-warfare agents is a record of the collaboration of a great many agencies and investigators. Without the development of machinery for the smooth exchange of information and the integration of the studies of many different groups, it would have been impossible for the participating workers to adopt adequate and uniform experimental technics and to achieve the results detailed herein.

The investigators of the Committee on the Treatment of Gas Casualties of the National Research Council and the Committee on Medical Research were interested in all aspects of the work and were often contributors to these studies. However, the results obtained were in the truest sense the outcome of the co-ordinated work of many groups, including not only the Committee on Medical Research but also the numerous official and non-official agencies, both foreign and American. The general result of this collaborative effort was the development of practical and useful methods for comparative evaluation of the efficacy, irritancy, sensitizing capacity, and stability of vehicles and compounds applied to the skin.

Much of the fundamental information obtained cannot be detailed here, but it may be stated that new insight was gained on such vital topics as the function of enzyme systems of the skin, the mechanism of blister formation, and the routes by which agents penetrate the skin. There were also many increments to the knowledge of the mechanisms of skin repair and epithelialization and of the factors influencing allergic skin sensitization (Fig. 83). It was, for example, established that the presence of damaged cutaneous tissue increases the susceptibility to sensitization and that locally applied agents can produce either local or generalized skin sensitization or systemic sensitization, as well as combinations of these.

The particular results were as follows: the selection of gas-protective [1]

---

[1] In this report the term "protection" is used to describe the beneficial effects obtained by agents used before exposure to a vesicant. The term "decontamination" is used to describe any process that either removes or inactivates a vesicant, or exerts both actions, at a surface that has been exposed. The term "treatment" signifies some step other than protection or decontamination taken to obtain a beneficial effect after the vesicant has penetrated the surface of the skin.

and decontaminating or treatment ointments superior to those previously available (that is, the present chloroamide-containing ointments and BAL-containing, specific anti-arsenical ointments); the formulation of precise directives for prevention and treatment of chemical-warfare injuries of the skin, in factories and arsenals and in military and civilian personnel; and the development of better preparations for the local treatment of burns and removal of slough in third-degree burns.

From the beginning of World War II until after D Day, it appeared that our enemies might launch large-scale attacks with blister gases. It was obvious that in case of such attacks each person would have to "help himself and carry on." It was therefore imperative to provide everyone with adequate means for treatment and for protection against these agents. In this situation the first place was accorded to studies that were expected to lead to the discovery of the best agents for protecting the skin and for treating such skin injuries as might result from exposure to the vesicant gases.

During the earliest phases of the work, high hopes were entertained that knowledge of the mechanisms by which the blister gases produced skin damage might lead to more effective protective and treatment devices. These hopes were richly realized in the discovery and introduction of BAL for protection against and treatment of injuries from lewisite and other arsenical gases. The evidence that mustard gas produced its damage through poisoning certain enzyme systems in the skin or through nucleotoxic action on cells about to undergo mitotic division, or both of these, as well as the discovery of many other significant facts regarding the pathogenesis of mustard-gas injuries and of blister formation in general, did not lead to specific means of preventing or reversing the damage from mustard gas. Instead, early in 1943 all investigators agreed that the changes were probably irreversible and that new specific treatment agents were not likely to be discovered in time to be useful in the war. It was therefore decided that the most energetic efforts should be made to develop and supply the best and most practical protective and decontaminating measures based on the earlier nonspecific methods.

The efforts in this direction fell into two natural divisions: the development of wearing apparel, protective gloves, and masks for the mechanical protection of the skin from contamination (these were largely the problems of the Army, Navy, and National Defense Research Committee); and the development of materials, principally ointments, to be applied to otherwise uncovered areas of skin as protectives and to be used to inactivate liquid mustard gas at the surface of the skin or to decontaminate objects.

As a basis for the inaugural studies, there remained from the previous war and from desultory researches of the inter-war period data showing that certain chlorine-containing compounds were the best-known chemicals for rapidly destroying mustard gas on the surface of the skin and on objects.

In 1943 the Army had on issue one chlorine-containing ointment, the Navy another, the British a third type, the Canadians a fourth, and our other allies (and also our enemies) still other agents. Despite the efforts of many investigators, time-consuming tests, and many and sometimes acrimonious conferences, the available information for establishing the relative merits and demerits of these ointments remained inadequate.[2]

In addition to these doubts concerning the relative values of different preparations, there were several incontrovertible and unfortunate facts. For example, General George Patton had cabled from the African Theater that the Army issue ointment should be immediately condemned and withdrawn from his area of command, it being itself a dangerous skin irritant and a regular producer of dermatologic casualties when used under conditions of desert warfare.

THE SUBCOMMITTEE ON ANTI–MUSTARD GAS OINTMENTS

It was at this time (1943) and in this situation that the Committee on the Treatment of Gas Casualties formed the Subcommittee for the Further Study of Protective and Decontaminating Ointments for Use Against Mustard Gas. The first step of this new subcommittee was to convince the various groups that it was not to be a special pleader for any one ointment or any one agency. It would seek only to help in developing objective and controllable technics of study, and to correlate and evaluate the results when such technics were uniformly employed by all the groups concerned.

The function of the Subcommittee was further confined to the biologic and in vitro testing of the available agents and of any other promising materials that might be submitted. The development, preparation, and procurement of active ingredients and ointment formulas were the tasks of other agencies, such as the National Defense Research Committee and its contractors, the Chemical Warfare Service of the United States Army, and the United States Naval Research Laboratory. In addition, numerous individuals and many nonofficial groups submitted chemical compounds and composite formulas for testing.

The co-ordinated programs of these groups and the Subcommittee permitted the rapid preparation and supply of the active chemical compounds, the formulation and manufacture of pharmaceutically desirable ointments containing the selected compounds, proper packaging, and the simultaneous study and control of each product by means of standardized biologic, phys-

---

[2] This statement is borne out by the 1943 reports of a special committee appointed to review all the data, including those of the most recent competitive tests at Gadston, Alabama, in which the Army and the Navy each attempted to demonstrate the value of its own ointment. After careful review and deliberation, the committee came to the decision that no selection was possible on the basis of the available data.

FIGURE 83. Effect of 4 weeks' application of liquid lewisite.

FIGURE 84. Typical experiment for evaluating comparative treatment efficacy of three different agents against blister gas. (Photograph at 8 days.)

FIGURE 85. *Effect of treatment with BAL 45 minutes after exposure to liquid lewisite. (Photograph at 2 days.)*

FIGURE 86. *Test of treatment efficacy of BAL 30 minutes after exposure to liquid lewisite. (Photograph at 3 days.)*

ical, and chemical tests. After preliminary screenings of a large number of materials, about twenty anti-mustard gas preparations were subjected to partial or complete investigation according to standardized methods.

TESTS FOR RELATIVE PROTECTIVE, DECONTAMINAT-ING, AND TREATMENT EFFICACY AGAINST MUSTARD GAS

While tests on goats, guinea pigs, rabbits, and other laboratory animals might give a rough idea of effectiveness, the reactions of the skin vary so greatly from species to species that it was soon found that the only constantly reliable test object was man. Eventually all promising preparations were tested on a series of volunteers. In devising the standard tests it was necessary first to ascertain optimum quantitative exposures to the chemical-warfare agent, optimum time intervals, and optimum quantities of the protective and treatment materials under study, in order to bring out as sharply as possible any differences in efficacy.

In controlled tests in each subject the damage at symmetrically situated sites was compared. The exposure to the vesicant, the time intervals, and other factors were held constant for all sites (Fig. 84). A known standard anti-gas agent was applied to one site (positive control) and the new anti-gas material to another. Often a third site, to which no protective or treatment agent was applied, served as a negative control. Since the same standard positive control was always used, it was possible to compare indirectly a large series of preparations studied at different times. Those that appeared to be the most promising were then directly compared in a series of volunteers. These tests as well as those described below were carried out in uniform fashion. The records of all tests were kept, and the results were pooled and evaluated on a statistical basis.

The principles of some other tests will be included here since they or their modifications may well have many uses outside of chemical warfare or wartime medicine.

TESTS FOR RELATIVE IRRITANCY TO HUMAN SKIN

Test I. Each group of investigators applied each agent under investigation in equal amounts (about 0.5 cc.) to equal-sized areas in the middle of the flexor surface of the forearm in a series of at least ten volunteers. Inunctions were applied for thirty seconds with a gloved finger three times daily on three consecutive days or until persistent irritation appeared at the site.

Test II. This was carried out simultaneously with Test I and with the same procedure as in that test, except that the agent was applied to the retroauricular area.

Test III. Each group of investigators applied each agent to a minimum of ten men, each man receiving an application of about 2 cc. to the groins, scrotum, and inguinal area by gentle rubbing for thirty seconds. The applications were made once every day for seven days or until irritation had appeared.

During the test period all conditions (diet, clothing, bathing restrictions, exercise, other activities, and so forth) were kept as nearly identical as possible. Uniform standards for reading and recording the degrees of irritancy and the uniform use of conventional symbols (o to $+++ +$) were adopted by all participating groups.

In Tests I and II in each series, one agent under investigation was applied to one arm and one retroauricular area of each volunteer, and the standard control material with which it was being compared was simultaneously applied to the other arm and the other retroauricular area. In Test III only one agent could be used on each man.

Eventually the poorer — that is, most skin-irritating — preparations had been screened out in this manner. By December 1943, Tests I and II were intensified and made to approach more closely the conditions of expected use in warfare. From then on, the frequency of applications was increased to once every hour for nine consecutive hours or until prohibitive and persistent irritation appeared.

The ointments finally selected passed the intensified Tests I and II, as well as Test III.

TESTS FOR SENSITIZATION INDICES

Sensitization indices were established by the following procedures, which were based on the earlier recommendations for studying BAL and related thiol compounds. Each participating group tested each agent on a minimum of thirty men. About 0.25 cc. of the agent was rubbed into the flexor surface of the forearm fairly vigorously once daily for a maximum of fourteen days or until a persistent erythema or greater reaction appeared. At three weeks after the beginning of the inunctions, provided no visible reaction remained at the site of the applications, the agent was applied once more to the previously inuncted site and to a new, previously untreated site on the other arm. At the same time, patch tests and scratch tests were performed with the agent and with any other substances that were of interest because of their chemical or immunologic relationships. The back or some other previously unexposed skin area was used for these tests. All skin tests were performed in the orthodox manner and read in the generally accepted and uniform fashion.

In each test series, at least ten subjects who had had no known previous exposure to the agent under investigation or to known related compounds

were used as controls. They were tested by a single inunction, by patch tests, and by scratch tests in order to rule out primary irritancy of the preparations.

Tests for Heat and Cold Stability of Agents

An adequate number of samples of each ointment were packaged in the selected containers (in this instance tubes) and subjected to three types of aging: standard shelf storage (at room temperature); storage in an oven at 50° C. for one month; and storage on alternate days at − 40° C. and + 50° C. for one month. At given times the tubes and their contents were examined by physical and chemical measures and selected ointments were again subjected to biologic tests for efficacy.

Tests for Systemic Toxicity on Percutaneous Application

Two ounces of ointment was applied as evenly as possible on each of a series of volunteers every day for seven consecutive days, covering the back, chest, shoulders, and upper arms but excluding the axillas. Every morning the remnants of the previous day's application were washed off before re-application. Complete physical examinations were done before the first inunction, including a complete urinalysis, a blood count, and any other special tests, such as those for methemoglobin and icteric index, indicated by the composition and suspected or recognized by-effects of the material under test. Examinations for evidence of dermatitis, cyanosis, and so forth were made daily before the next application. On the second, fourth, and seventh days of the experiment, two hours after the inunction, the blood count, urinalysis, methemoglobin measurements, and other indicated tests were repeated.

It was of course recognized at the outset that the selected test procedures would give only comparative data and could be expected only to range the tested ointments in their relative order of merit in regard to each of the properties concerned. The tests were not expected to be conclusive as to the absolute value of any of the ointments under conditions of actual use. It was indeed repeatedly emphasized that only extensive field trials and finally field use under the varying conditions of simulated or actual combat could yield conclusive data on the absolute usefulness and disadvantages of any preparation. However, it was considered logical that the ointments that proved best in the adopted tests (including those for decontaminating efficacy against liquid mustard gas, for effectiveness and duration of protective capacity against mustard-gas vapor, and for low irritancy and low skin-sensitizing potential) had, by and large, the best prospects of showing up well under field conditions.

ADVANTAGES AND LIMITATIONS OF SELECTED ANTI-MUSTARD GAS OINTMENT

On the basis of the results of the above program, the Army and Navy, and later the Canadian Army, selected and issued gas-protective ointments of the

TABLE I

Comparison of Approved Army and Navy Ointments against Mustard Gas As Selected and Issued Before and After the Completion of the OSRD Program

	Approximate Percentage of Active Chlorine	Persistence of Protection	Decontamination of Liquid	Lack of Irritation	Stability on Storage	Lack of Harm to Tubes	Acceptability to Users
	%						
Early Army, M–1	7.4	++	+++	0	(+)*	0	+
Later Army, M–4	4.4	++	+++	0	(+)*	0	+
Early Navy, S–461	15.0	++	+++	+	+++	+++	++
Later Navy, S–461	5.0	++	+++	++	+++	+++	++
Present issue ointment†	7.5	+++	+++	+++	++*	+++	(+)

* Estimated on the basis of other data and not resulting from direct studies with the actual formula.

† NC–III-type ointment, designated as M–5 by the Army and S–330 by the Navy, and both containing S–330 as the active ingredient.

type that showed a significant superiority throughout. Table I compares the desirable and undesirable properties of the older and newer issue preparations.

As it will be seen the new S-330 ointment selected by the Army and Navy [3]

[3] There are minor differences in the amounts of pigment, cellulose acetate butyrate, and titanium dioxide in the Army and Navy issue ointments, but the preparations are essentially alike, and both contain S–330 as an active ingredient.

Army M–5 (approximately 7.75 per cent active chlorine)

	%
S–330	25.0
Cellulose acetate butyrate	4.0
Titanium dioxide	9.0
Triacetin	52.0
Magnesium stearate	9.0
Pigments, mixed	1.0

(Chlorine — fast green pigment, 20%)
(Chlorine — fast brown pigment, 80%)

was a great improvement over the former Army M–1 and M–4 ointments in respect to lack of irritancy, increased stability, and lack of harm to tubes, and a moderate improvement in regard to persistency of protection. It also constituted a distinct improvement over the former Navy S–461 ointments in regard to lack of irritancy.

Later observations and experience during field trials revealed the following characteristics of the new ointment. It is green and sticky and therefore disagreeable for use in the field. It not only smudges eyepieces of the gas mask but etches the plastic of the present eyepieces, thus permanently reducing their transparency. A further drawback lies in the fact that at points of sweating and friction, such as the collar line and the edges of sleeves, it tends to roll and to peel off, leaving the areas unprotected.

In weighing the advantages and disadvantages of the new ointment, it is to be realized that it still suffers from the basic drawbacks inherent in all chlorine-containing preparations. They are capable of destroying only the portion of the vesicant that has not yet entered the living tissues of the skin. For this reason they are largely ineffective if used more than two to five minutes after exposure to liquid vesicants and are of no practical value in treating the effects of exposures to vapor, in which the vapor generally penetrates almost as quickly as it impinges on the skin. An additional drawback of this ointment, and of all chlorine-containing and oxidizing compounds, is that they do not satisfy the military needs for a universal or polyvalent protective and decontaminant; for example, the selected ointment has no significant effects against any of the nitrogen mustards. On the other hand, when applied under the same conditions as when used against mustard gas, and at the same time intervals after exposure, the selected ointment inactivates liquid arsenical vesicants (lewisite and the like) on the surface of the skin. Thus, the ointment is effective when employed in the early decontamination of liquid arsenical vesicants or of mixtures of liquid mustard gas and arsenical vesicants. However, when more than two to ten minutes has elapsed after exposure to the arsenical, BAL, which penetrates into the tissues and actually removes the toxic agents from the cell, is a much more effective anti-arsenical agent. A further great disadvantage of the chlorinating ointment is that it cannot be used in the eyes or on the lids, since it produces severe irritation and damage.

In view of all these shortcomings, there is obviously still a real need for the discovery of improved measures and new approaches. Nevertheless the development of this new, relatively stable chloroamide-containing ointment, which is of comparatively low irritancy yet confers relatively long periods of protection against field concentrations of mustard-gas vapor, constitutes a step forward. All the previously employed American ointments, and in particular the Army M–1 and M–4 ointments, were so irritating as to preclude completely their repeated application to human skin as protective

agents. The British ointments, while of low irritancy, were relatively unstable and of relatively short effectiveness as protectives.

Finally, mention should be made of two items that, although not connected with chemical warfare, have nevertheless been outgrowths of this program. First, the development of several relatively stable and relatively nonirritating yet effectively detoxifying chlorine-containing ointments has suggested the possibility of using these or analogous preparations as protectives against a large variety of occupational and other agents commonly causing contact dermatitis; for example, poison ivy. Second, the apparent success of the methods here developed for studying the relative irritancy, relative sensitizing capacity, and relative effectiveness of these chloroamide ointments suggests the wider usefulness of such procedures for ascertaining the relative merits and demerits of other materials intended for application to human skin.

BAL OINTMENT PROGRAM

ARSENICAL VESICANTS

As stated, the chloroamide ointments inactivate liquid arsenical agents, such as lewisite, on the surface of the skin, in much the same fashion as they inactivate mustard gas. On the other hand, locally applied BAL follows the arsenical vesicant and penetrates into the tissues, where it combines with it to form a relatively stable and nontoxic compound and thus reverses the trend of the tissue injury. This explains why BAL can succeed in actually reducing the damage, even when applied locally as long as thirty to sixty minutes after exposure to the vesicant (Figs. 85 and 86).

Because of these facts, as soon as BAL was available in sufficient amounts it became essential to supply military personnel with BAL preparations for use on the skin. In order to develop suitable ointment vehicles and packages, the so-called BAL Ointment Program was set up as a joint project of the Committee on Medical Research and the National Defense Research Committee. The contractors of these agencies, together with Army and Navy research workers, set about the development of a suitable, stable, and effective BAL-containing preparation that would be useful for both skin and eyes. Since technical considerations made it highly unlikely that the enemy could use effective concentrations of arsenical vapors, the BAL ointments, in contrast to the chloroamide ointments, were intended primarily for treatment rather than as prophylactic or protective coverings.

In biologic tests for evaluating the relative merits of more than seventeen different BAL-containing preparations, optimum concentrations of vesicant, optimum concentrations of BAL, and optimum time intervals had to be fixed in order to bring out as sharply as possible any differences in effective-

ness. With these optima each new preparation under consideration was com-
pared with a standard control BAL preparation for effectiveness against
liquid lewisite on the skin and in the eyes of rabbits. In this way each BAL
preparation could be indirectly compared with any other member of the
series. The ultimate contenders were then directly compared with each
other, not only on rabbits but also on the skin of volunteers.

In addition to these tests for efficacy, extensive studies were performed on
volunteers to ascertain the skin irritancy and skin-sensitizing potential of the
different preparations and their general toxicity on percutaneous application
to large areas.

Further tests were carried out on irritancy to the human eye, stability on
storage and under variable temperatures, and the effects of and on different
types of containers. After artificial aging in the selected containers, the better
preparations were again tested on animals and men for the above specified
beneficial and potentially harmful effects. As in the chloroamide program,
these studies were conducted by several groups working under different
agencies and at different places but with uniform technic. The final results
when assembled permitted certain conclusions and inferences, which may be
summarized as follows:

(1) The external application of BAL is a far more effective treatment for
skin contaminated by arsenical vesicants than is the external application of
any non-thiol-containing substance with which BAL has been compared.

(2) The preceding external application of BAL in certain vehicles will
effect some protection against skin damage by arsenical vesicants. For this
purpose some vehicles proved vastly superior to others. For example, K–Y
Jelly has been shown to be markedly superior as a vehicle to both vanishing
cream and a mixture of zinc oxide, talcum, glycerine, and water.

(3) The conditions of temperature and humidity, the type of container,
the concentration of BAL, its manner of application, and in particular the
type and ingredients of the vehicle in which it is incorporated can exert dis-
tinct influences on the stability, therapeutic efficacy, protective capacity,
irritancy, and skin-sensitizing potential of the agent.

(4) Adequate methods and standards for accelerated aging and for the
testing of vehicles and containers for BAL have been developed. These
methods include physical, chemical, and biologic procedures. The vehicles
studied comprised many water-soluble gum bases, grease bases, vanishing-
cream-type bases, and nonaqueous water-soluble bases.

(5) The evaluation of these preparations by standard methods has led to
the formulation and development of several relatively stable and effective
BAL ointments for use on the skin and in the eyes. Certain of these oint-
ments have been accepted and issued for use by the British and American
armed forces. The accepted American ointments are those given in the
following lists.

Navy Issue Formula 13	%	Army Issue Formula 14	%
Peanut oil	36.95	Boric acid	1.898
Lanolin, anhydrous	8.0	Carbowax 4000	7.592
Cetyl alcohol	10.0	Carbowax 1500	47.45
Glyceryl monostearate	10.0	Ethylene glycol	37.96
White petrolatum, soft	25.0	Isoascorbic acid	0.05
Benzyl benzoate	5.0	Thiamin hydrochloride	0.05
Mixed tocopherols, 40%	0.05	BAL	5.00
BAL	5.0		

(6) The experimental data in sum indicate that, when used according to the directives, the BAL ointments selected represent an optimum therapeutic index — that is, the best obtainable effectiveness in early treatment of eyes and skin contaminated by arsenical vesicants — combined with a low irritancy to the eye, a negligible skin irritancy and skin-sensitizing potential, and a practically negligible systemic toxicity.

(7) Clinical experience, although as yet somewhat meagre, confirms the above experimental findings.

(8) BAL is ineffectual as an early treatment agent against mustard gas. Against mixtures of liquid lewisite and liquid mustard it exerts the expected beneficial effects in relation to the lewisite component of the mixture but is ineffective against the mustard-gas component.

(9) When administered systemically in large amounts shortly before exposure to liquid lewisite, BAL is capable of somewhat modifying the skin damage produced by the vesicant.

(10) Experiments indicate that the external application of BAL will not suffice to combat the effects of massive exposures and systemic poisoning by arsenical vesicants. In such cases the external application must be supplemented by systemic administration of BAL. Extensive toxicologic and pharmacologic experiments in laboratory animals and toxicity tests in man have succeeded in establishing the present optimum vehicles, concentrations, and dosage schedules for such systemic administration in man.

(11) The aggregate results of all studies to date show that BAL is by far the best available anti-arsenical agent. The principal practical problems of its employment on skin and eyes contaminated by arsenical vesicant warfare agents have been elucidated, and the principal practical difficulties in the way of its military and civilian employment have been overcome.

LATE TREATMENT OF SKIN INJURIES

While the preceding pages mention some of the problems and progress in the prevention and early treatment of blister-gas injuries — that is, before the definitive clinical lesion is present — the following summarizes the status

of local external treatment of the fully developed cutaneous injury from the vesicant agents of chemical warfare.

Many hundreds of controlled tests were performed on a great number of laboratory animals and volunteers. In these tests symmetrically situated skin sites in the same volunteers were burned with measured equal quantities of vesicant agents. In general one such site was treated with the preparation under investigation, one left untreated, and others treated, for comparative purposes, with other agents or with standard control preparations.

The net results may be briefly stated as follows:

(1) Vesicles or bullae need not be opened unless tense or painful. In this case evacuating the contents and leaving the blister top in place is indicated.

(2) Vesiculating and second-degree vesicant burns require no more than a dry dressing with slight pressure or wet compresses and lotions, applied as for dermatitis from other vesicants (poison ivy and so forth).

(3) Deep necrotic burns should be treated locally and systemically like third-degree thermal burns.

(4) Petrolatum dressings tend to macerate and produce superficial pyodermas of the surrounding skin.

(5) Petrolatum containing 0.33 per cent silver nitrate forms a good local dressing, helps to keep the wound clean, and does not generally irritate or sensitize.

Sulfonamide-containing creams tend to keep down infection and do not significantly retard healing but sensitize a fair proportion of subjects, both cutaneously and systemically. Different sulfonamides have different sensitizing potentials. Thus, sodium sulfadiazine ointment on repeated application to the wounds of human subjects sensitized the skin of 57 per cent; sulfanilamide sensitized 22 per cent; sulfathiazole sensitized 7 per cent; and sulfadiazine sensitized 5 per cent. It will be seen that this incidence of sensitization corresponds to the order of the drugs' solubility in water. (All sulfonamides were incorporated in 5 per cent concentration in the same type of water-miscible cream.) Subjects who developed skin hypersensitivity through topical application showed a much higher than normal incidence of rashes and headaches, nausea, dullness, and dizziness when given sulfadiazine by mouth (6 gm. in divided doses over twenty-four hours).

When evaluated by careful comparison with the courses of similar burns in symmetrically situated sites in the same persons, it could be shown that none of the following agents accelerated closure, epithelialization, and healing of the ulcers resulting from third-degree burns:

Pressure dressings	Enzymol-hydrochloric acid solution followed by
Sulfonamide ointments	marfanil-streptomycin solution (Howes)
Petrolatum dressings	Penicillin solution or ointment
Tissue extracts	Three per cent vioform ointment
	Boric acid ointment

Spraying with horse serum, followed by exposure to a heat lamp, apparently somewhat accelerated healing, but folliculitis complicated this form of treatment.

The only tested measure that regularly and significantly accelerated the rate of healing of the treated burns over the control burns was early débridement, with gentle, clean removal of the necrotic tissue.

The use of 0.1 M pyruvic acid in starch paste, followed by sodium sulfadiazine cream, for chemical débridement shortened the healing time of third-degree mustard-gas and third-degree thermal burns by as much as one third (Table II).

TABLE II

Effect of Pyruvic Acid in Starch Paste on Third-Degree Burns

No. of Volunteers	Type of Burn	Average Healing Time (days) with Pyruvic Acid Paste	Treatment Used on Symmetrical Lesion	Average Healing Time (days)
11	Thermal	20.2	No treatment	30.9
10	Thermal	19.4	Blank starch paste	24.0
13	Chemical	30.0	Blank starch paste	39.7
4	Chemical	31.5	5% sodium sulfadiazine cream	42.8
6	Chemical	38.1	0.33% silver nitrate in petrolatum	49.0
5	Chemical	31.1	5% sodium sulfadiazine cream	43.8

Since, however, the pyruvic acid was unstable and difficult to obtain in sufficient quantity, and since the starch paste had to be freshly prepared at each dressing and was unstable and impractical, efforts were made to develop more practical and stable combinations of acids and gels.

In these efforts symmetrically situated chemical (and later thermal) burns were produced on several hundred volunteers. The efficacy of a large variety of different chemical débriding preparations was compared with that of the standard 0.1 M pyruvic acid in starch paste. Many interesting observations were made during the course of these studies, and new methods were developed for the preliminary in vitro evaluation of different vehicles. In the last analysis the most promising preparations were always studied for efficacy and irritancy in rigidly controlled tests on man, in a manner somewhat analogous to that already described for the chloroamide ointments and for the BAL ointments.

After extensive and time-consuming studies with many acids and vehicles, seven more stable and practical preparations were developed and shown to be approximately as effective as pyruvic acid in starch paste in removing slough and shortening healing time. Three examples of such gels follow:

Methyl cellulose-water gels with 0.1 M pyruvic or
phosphoric acid

Pyruvic acid ....   6.9 cc.
Methyl cellulose (25 cps.) .... 170.0 gm.
Water .... 821.0 cc.

Phosphoric acid ....   6.74 cc.
    (85% — 1.71 sp. gr.)
Methyl cellulose (25 cps.) .... 170.0 gm.
Water .... 818.5 cc.

K–Y Jelly (Johnson and Johnson Co.) with 0.4 M pyruvic or
phosphoric acid

Phosphoric acid ....   27.0 cc.
K–Y Jelly .... 1000.0 gm.

These gels still had the inconveniences of bulk and unknown stability. Dry acid-containing powders were then prepared, which, on the addition of correct amounts of water, rapidly formed gels suitable for acid débridement of burns. In biologic tests on symmetrical human burns, the gels formed by some of these powders proved to be almost equal to pyruvic acid in starch paste in débridement and acceleration of healing and superior to it in their low irritancy and slight maceration of the surrounding skin. The following are examples of the most satisfactory powders tested:

Pyruvic acid ....   66.0 gm.
Methyl cellulose .... 500.0 gm.
Mix 1 part of powder with 13 parts of water. pH of gel is 2.0.

Pyruvic acid ....   36.8 gm.
Methyl cellulose .... 300.0 gm.
Sorbitol ....   60.0 gm.
Mix 1 part of powder with 10 parts of water. pH of gel is 2.2.

Phosphoric acid .... 20.0–30.0 gm.
Methyl cellulose .... 300.0 gm.
Sorbitol ....   60.0 gm.
Mix 1 part of powder with 10 parts of water. pH of gel is 2.0 to 1.79.

As a rule the débriding applications were begun on the third or fourth day after thermal burns and on the seventh day after mustard-gas burns. This interval was allowed to elapse in order to permit some diminution of the early exudation. Fifteen to 20 cc. of the vehicle containing the acid-débriding agent was then applied liberally to the lesion and was immediately covered with gauze. One or two layers of vaseline gauze were next applied, followed by ordinary gauze bandage, which was held in place by wide strips of elastic adhesive bandage. This dressing was made more occlusive by sealing the

edges and seams with ordinary adhesive plaster. The applications were re-
newed daily until débridement took place or until a prohibitive degree of
irritation developed in a particular subject. On the average, débridement
required a total of four to five days for thermal burns and eight to ten days
for chemical burns. When the débriding agent was discontinued, both
lesions were treated identically. The post-débridement treatment was applied
daily under a gauze dressing until epithelialization was complete.

The pyruvic acid-starch paste method and the practical modifications of it
here described appear to carry promise in the civilian treatment of third-
degree burns and other necrotic, torpid, and ulcerating defects. The experi-
mental results and preliminary clinical experience indicate that this method
not only reduces healing time but is capable of rapidly producing a clean
granulating surface ready for grafting. Moreover, the incidence of local in-
fection appears to be reduced by the acid treatment, and the scars left by the
acid-débrided wounds are softer, flatter, and more pliable, although some-
what larger, than those from similar wounds treated by older measures.

SUMMARY

The wartime studies aimed at finding better means for preventing and
treating skin injuries from blister gases have had some practical success and
have resulted in the improvement of ointments for protection, decontamina-
tion, and treatment. On the theoretical side, new knowledge has been gained
regarding the mechanisms of blister formation and cutaneous tissue damage,
the enzyme systems of the skin, and specific sensitization processes. More-
over, measures for local treatment have been improved and a method has
been developed for chemical débridement and accelerating healing of third-
degree burns and necrotic wounds.

Last, and perhaps most important, the urgencies of war have led to the
development of methods that should in the future prove useful in the evalua-
tion of vehicles and materials for topical applications, as well as for rapid
comparisons of the relative efficacy, irritancy, sensitizing potential, and
systemic toxicity of a great variety of agents intended for use on the skin.

THE EFFECTS OF TOXIC CHEMICAL AGENTS ON THE EYE AND THEIR TREATMENT

JONAS S. FRIEDENWALD AND W. F. HUGHES, JR.

THE INFORMATION on chemical burns of the eye acquired prior to 1941 consisted largely of clinical and histologic descriptions of the course of the lesions and of therapeutic measures designed to remove as much of the corrosive agent as possible by means of irrigations and neutralizing solutions or to obviate secondary complications. Such procedures had been worked out for mustard gas from experiences of World War I and for alkali burns from civilian accidents. Knowledge of the mechanisms by which certain chemicals damaged the cornea was extremely scanty, owing in large part to ignorance of normal corneal physiology.

In 1941, it became apparent that additional studies were required along two general lines. First, it was thought necessary to substantiate or improve existing routines of treatment for mustard gas, to study the characteristics of certain new war gases such as lewisite and the nitrogen mustards, to work out the optimum conditions for using the new British antidote BAL, and to apply newer therapeutic agents such as the sulfonamides and penicillin in an effort to prevent secondary complications after chemical injury. Second, more fundamental studies were needed of the alterations produced by toxic chemicals in the chemistry and physiology of the cornea. Through such long-range studies, it was hoped that light would be thrown on the problem of suppressing or ameliorating lesions produced by chemical injury.

TREATMENT OF MUSTARD–GAS BURNS OF THE EYE

To determine how long after exposure an ideal surface decontaminating agent or a 100 per cent effective penetrating agent could be expected to be ·beneficial, a study was made in rabbits of the rate of penetration and persistence of mustard in the cornea. In order to approximate field conditions as closely as possible, either the mustard was applied as a small droplet on the cornea, immediately followed by closure of the lids (the so-called "splash-and-blink" technic), or the eye was exposed to mustard vapor (see below for details of these methods). One minute after such exposures, little if any residual mustard could be transferred directly from the exposed cornea to a

normal cornea. The corneal epithelium and endothelium did not impede the penetration of the mustard through the cornea into the anterior chamber, iris, and lens, but radiophotographs of mustard with radioactive tagged sulfur showed that the anterior layers of the cornea held most of the mustard for weeks after exposure.

Two chemical technics were employed to determine how long free mustard remains in the cornea: extraction of mustard with cyclohexane-kerosene and iodometric titration after reaction with dichloramine-T, a process that does not extract mustard derivatives such as so-called "semi-mustard" (b-hydroxy-b'-chlorodiethyl sulfide); and extraction of mustard and probably certain toxic mustard derivatives with isopropyl alcohol together with colorimetric estimation of the mustard material by Johnson's DB 3 reagent. The half-life of mustard by the first method was found to be three minutes at 38° C. and thirteen minutes at 24° C., and by the second method to be nine minutes at room temperature. The mustard that becomes inextractable by these methods is probably combined irreversibly with the tissues. In favor of this view is the fact that the normal swelling of the corneal tissue when placed in water is inhibited by mustard at a rate paralleling the disappearance of free mustard. Such combinations of mustard with tissue have not been found to be reversible.

In summary, mustard disappears from the surface of the rabbit cornea within one minute after exposure, and within three to nine minutes half of the mustard becomes irreversibly attached to the tissues. Therefore, a penetrating antidote designed to combine with free residual mustard in the tissues would necessarily be beneficial for only a short period of time after exposure.

The chief methodological problems in the investigation of the value of various therapeutic agents for chemical injuries of the eye are the choice of animals for study, the production of a standard lesion of controlled severity in the experimental animal, and the numerical estimation of the severity of the ocular reaction so that the treated and untreated eyes can be compared statistically.

The rabbit has proved to be the most satisfactory animal for the large-scale testing of therapeutic agents. In general, the ocular reactions to vesicant agents are somewhat severer in albinos and young animals and less severe in black rabbits. Since variation in the severity of the reaction produced by the same dose of vesicant in the eyes of different animals is greater than that between eyes of the same animal, crucial comparisons are made in the same animal.

The production of a satisfactory standard lesion is dependent on the following: accurate dosage, a standard technic in application of the vesicant to the eye, and selection of a dose large enough to give a severe, reproducible lesion and yet not large enough to mask the effect of a therapeutic agent

of only moderate efficiency (Fig. 87). Many technics were tried during the early phases of the work, and the following were eventually used
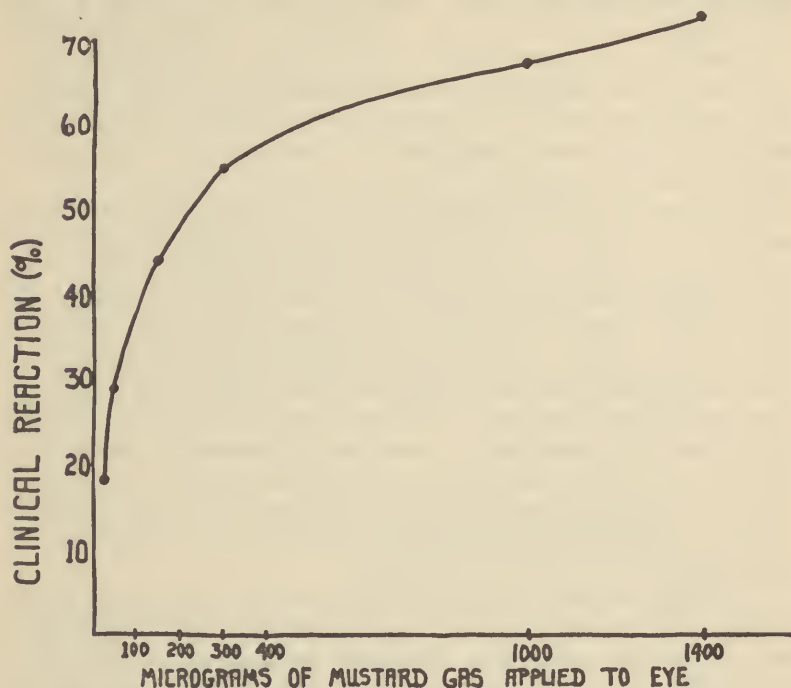
FIGURE 87. *Chart showing relation between dose and severity of clinical reaction produced by an instillation of liquid mustard into the rabbit's eye.*

by most investigators because of their ease, reproducibility, and similarity to the type of exposures that might be met in chemical warfare. Liquid vesicant agents were delivered from a 0.25-cc. tuberculin syringe, and a 26-gauge hypodermic needle with the bevel filed off to a square end was used, the plunger being activated by a micrometer screw. Volumes as small as 0.1 cm. could be expelled with an accuracy of 20 per cent, although about 50 per cent of the droplet remained adherent to the needle after being touched to the cornea. With the rabbit under general anesthesia, the droplet of vesicant was applied either on the center of the cornea or, oftener, on the upper limbus, the latter eliciting somewhat more severe reactions. The lids were closed by the splash-and-blink technic, and treatment was instituted at varying intervals thereafter. Excellent standard lesions were also obtained from the vapor of vesicant agents by using a vapor chamber designed to expose only a single

eye. It consists of a small cylindrical glass chamber 2 cm. in diameter and 4 cm. deep, surrounded by a water jacket so that the temperature can be maintained constantly at 21 to 25° C. An excess of liquid vesicant is placed in the bottom of the chamber, and a filter-paper fan helps to maintain an even distribution of vapor. The eye is protruded between the lids and inserted over the mouth of the chamber for an exposure of a stated period of time, usually fifteen seconds to one minute. Thus it receives an exposure to saturated vapor at constant temperature, the dosage depending on the duration of exposure.

For the accurate study of toxic and therapeutic effect, it is essential to have some measure of the severity of the clinical reaction. A numerical scale can be worked out in which a grade is given to each significant symptom in the ocular reaction, more points being given to more important symptoms. The selection of the symptoms to be recorded and the weighting of the different signs requires judicious care and may be readily altered depending on the nature of the problem under investigation. Thus, conjunctival discharge is an important index when the role of secondary infection is being studied, but is of much less importance in connection with the search for an antidote. The sum of the points for all the symptoms in a particular eye gives the total grade of the lesion, and this may be converted to a percentage of the maximum possible lesion. Table I shows a representative chart designed for estimating the severity of chemical burns.

The validity for such a system of grading has been substantiated by the fact that the average recorded severity of the reaction of different sets of animals exposed to a graded dose of injurious agent forms a smooth curve, which is, however, not linearly proportional to the dose. Independent readings by several experienced observers are in remarkably good agreement. For statistical comparison between treated and untreated eyes, the severity of the ocular reaction on any particular day can be selected (usually the fourth to the seventh day for antidote experiments). To reduce the error in daily fluctuations of readings, the sum of the maximal recorded severities of each symptom over the course of observation can be taken as an index of the acuteness of the reaction — the so-called "maximum ocular reaction." The residual corneal opacity at a fixed date after exposure furnishes an index of the residual visual damage. Such figures can be compared statistically by calculation of the standard deviation from the mean values of the treated and untreated groups of eyes.

As stated previously, the possible range of usefulness of even the best antidote conceivable at present for mustard is limited because of the rapid penetration of mustard into the cornea and its combination with the tissue within five to ten minutes after exposure. No substance has been found that will remove mustard from its combination with proteins without destroying the proteins. Decontamination after exposure to vapor is therefore impossible

Table I

System for Grading the Severity of a Chemical Burn of the Eye

Maximum Grade

Corneal Symptoms:
  Opacification (density × area, 4 × 4):   16
    Easily detectable   1
    Blurs pattern of iris   2
    Blurs pupillary outline   3
    Complete   4
      For corneas with opacities of different intensities in dif-
      ferent areas: e.g.,
      · Area 1 — Density × area   4 × 1 = 4
        Area 2 — Density × area   3 × 3 = 9
        Total grade for corneal opacity   13
  Vascularization: 3
    Involving less than ½ circumference of limbus   1
    Involving more than ½ circumference of limbus   2
    Extending more than 3 mm. into cornea   3
  Ulceration: 4
    Stainable with fluorescein   1
    Shallow   2
    Deep   3
    Perforation   4 (100% corneal lesion regardless of
                            other values)
  Edema: 3
    Just detectable   1
    Marked   2
    Staphylomatous bulging   3
                                                                ___
      Total points for estimation of corneal lesion   26*

  Duration of corneal opacity: †   4
      1–3 days   1
      4–6 days   2
      7–13 days   3
      14 days and over   4
Conjunctival Symptoms:
  Redness   1
  Edema   3
  Necrosis (petechial hemorrhages and ischemia)   1
  Mucopurulent discharge   2
Iritis: 3
  Small pupil   1
  Congestion or thickening of iris   2
  Exudation   3
                                                                ___
      Total points for estimation of ocular lesion   40 (100%)

* In any case where the cornea was perforated, the lesion was counted as of 100 per cent severity.

† The record on duration of corneal opacity should be included only in the final tally.

because of the latent period of at least two to six hours after exposure before the onset of symptoms. It is theoretically possible, however, to accomplish partial decontamination if the subject is immediately aware of a mustard splash.

Following exposure to mustard vapor, immediate lavage is contraindicated because experimentally it is without therapeutic value, because any attempt to irrigate the eyes of a gassed soldier in the presence of vapor delays putting on the gas mask and may allow serious pulmonary damage to occur, and, lastly, because only a small percentage of vapor burns produce any significant permanent damage to the eye. Following contamination by liquid mustard, however, there is a period of about one minute during which irrigations with copious amounts of any nonirritating fluid have been shown experimentally to be of value. Field tests have demonstrated that a man can irrigate his own eye with a canteen sufficiently well to permit this procedure. Better results are obtained, however, if even an untrained companion performs the irrigation. Repeated irrigations later are contraindicated because they tend to loosen the corneal epithelium, but occasional lavage may be used to remove any accumulated discharge.

Immediate use of BAL ointment (see below under discussion of lewisite) has a slightly beneficial effect after exposure to mustard. Because of the somewhat irritating nature of the ointment and its minimal benefit, it is not recommended unless there is evidence of an associated lewisite burn; for example, an odor, an immediate stinging sensation, or blepharospasm.

Although little hope was held that an antidote could be found that would compete favorably with mustard already combined with the tissues, extensive studies were made to discover an agent that would reduce the severity of the lesion by combining with free mustard still present in the tissues. An ideal agent would be one that has a high reactivity for mustard, that is capable of penetrating into the tissues and within the cells reached by mustard, that forms a nontoxic reaction product with mustard, and that is itself nontoxic in therapeutically effective dosage.

The problem is further complicated by the fact that the anterior corneal surface presents a relatively impermeable barrier of special character, especially toward ionized substances. It seemed wiser to avoid this special problem at the outset and to discover whether or not there were any substances that if injected into the tissue simultaneously with mustard would prevent its damaging effect. To this end, a screening test was devised in which mustard and the potential antidote are mixed in aqueous lecithin emulsion and injected intracutaneously into the rabbit, with controls receiving mustard alone and the antidote alone. By means of a dilution series of the antidote, it is possible to determine the minimum concentration of antidote that is protective against the mustard in the mixture, and also the maximum concentration of the antidote alone that is nontoxic to the skin, the

amount of overlapping of the two dilution series of injections representing the so-called "therapeutic index."

Three groups of compounds were found that contained effective members. These were the dithiocarbamates $R_2NCSSNa$, orthoamino thiophenol,

 NH_2, and certain of its derivatives and analogues, and 2-5-dimercapto

thiodiazole, . All the compounds found effective in the
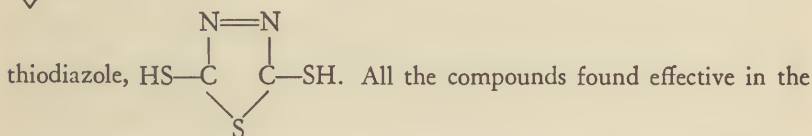
screening test contain sulfhydrils whose acid dissociation is below pH 7 and presumably have high competition factors for mustard. However, there are many other substances with equal or higher competition factors; for example, thiophosphates, which were found ineffective on the screening test. The presence of a nitrogen atom in close proximity to the SH group seems necessary for their effect; for example, no effect was obtained with chemically related substances such as the xanthates (R—O—CSSH), the monosubsti-

tuted trithiocarbamates (R—S—CSSH), and thiophenol, .
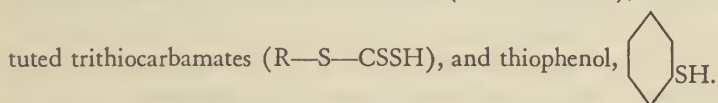
Therapeutic experiments on rabbits' eyes were performed using several of these substances. In one laboratory a 40 per cent aqueous solution of sodium diethyl dithiocarbamate was found to be effective in reducing the severity of the mustard lesion four minutes after exposure, but only slight effect was obtained with this compound in another laboratory using slightly different conditions of treatment. Two less toxic derivatives of orthoamino thiophenol were tested therapeutically on the eyes: 4-amino-3-mercapto-benzoic acid

(NDR–602), , and 4-amino-3-mercapto-benzoic acid ethyl ester
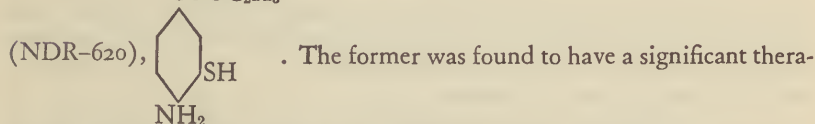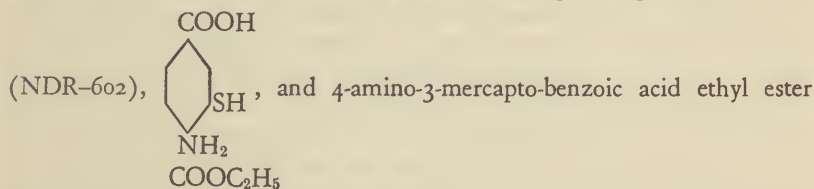
(NDR–620), . The former was found to have a significant thera-

peutic effect two minutes after instillation of 0.14–0.28 mg. of liquid mustard. This compound and especially NDR–620 in 2 per cent carbowax ointment

bases showed striking therapeutic effect against HN_2 or $(ClCH_2CH_2)_2NCH_3$ two minutes after exposure, but no benefit was obtained after exposure to HN_1 or $(ClCH_2CH_2)_2N$—C_2H_5, HN_3 or $(ClCH_2CH_2)_3N$, or lewisite.

No therapeutic effect was obtained by the use of various chlorinated compounds against liquid mustard burns; for example, dichloramine-T, chloramine-T, S–330, or S–461. In fact, the latter two ointments prepared for use on the skin make the ocular lesion much worse.

The role of secondary infection in ocular mustard lesions is uncertain. During the first twenty-four hours after exposure, cultures of the conjunctival sac are ordinarily sterile, after which time increasingly abundant conjunctival flora are obtained in animals. Local applications of penicillin and to a lesser extent of sulfonamides reduce the bacterial flora and by some investigators are thought to result in a slight but definite improvement in the corneal lesion, especially in mild and in very severe burns.

Penicillin solution or ointment in doses of 250 to 500 units per gram was found to be more effective than the various sulfonamides. Sodium sulfadiazine and sodium sulfacetimide were found to be the most satisfactory of the sulfonamides, the latter (albucid) possessing a slight analgesic effect. Most adequate penetration and maintenance of satisfactory sulfonamide levels in the cornea and aqueous were obtained by the use of 10 per cent rather than 5 per cent concentration, the use of drops or an oil-in-water type of ointment (for example, lanolin-petrolatum 50–50) every four hours, and the addition of the detergent Duponal M. E. Dry in 0.1 per cent concentration. Penicillin, sulfadiazine, and sulfacetimide did not inhibit mitotic activity of the corneal epithelium. However, the ointment bases *per se,* especially sulfacetimide, appeared to retard slightly the regeneration of epithelial defect involving the limbus and to result in some increase of the residual corneal scarring.

No reliable conclusions can be drawn from the experimental data cited concerning the importance of secondary infection in human eyes, the frequency of which may vary widely in different climates and individuals. However, a cornea denuded of epithelium with necrosis of the underlying stroma may easily develop an infected corneal ulcer regardless of the original cause of injury. Such abscesses, containing stainable bacteria, have been observed in animal eyes following chemical burns, and a few cases of panophthalmitis after mustard injury were reported in World War I. It is therefore probably advisable to instill penicillin ointment or possibly sodium sulfadiazine ointment into the eye every four hours, beginning twenty-four hours after exposure to any vesicant agent.

It is well established that local anesthetics interfere with regeneration of the corneal epithelium, both the processes of cell migration and of mitosis being affected. Comparative studies among the various anesthetics on rat

corneas have shown that in general anesthetics in ointments are preferable to those in solution, that 0.5 per cent phenacaine (holocaine) solution and ointment and 1 per cent nupercaine ointment had least effect on both mitosis and migration; that 2 per cent butacaine (butyn) ointment decreased mitosis markedly but had slight effect on migration; that 0.5 per cent tetracaine (Pontocaine Hydrochloride) ointment did not interfere with mitosis but delayed migration more than the others; and that 0.5 per cent cocaine solutions inhibited moderately processes of both migration and mitosis. In experimental mustard burns, the instillation of 0.5 per cent Pontocaine drops every four hours did not significantly affect the course of the lesion, although such a solution has moderately pronounced effects on both migration and mitosis.

LEWISITE BURNS

Exposure of the eye to either liquid or vapor lewisite produces immediately a sharp irritation and blepharospasm. Devastating ocular lesions are produced experimentally by doses of liquid lewisite as small as 0.1 mg., or exposure for eight seconds to saturated vapor at 23° C. Lewisite hydrolyzes immediately on contact with the moist surface of the eye, producing a superficial opacity from the liberated acid, followed by a progressive lesion of the cornea because of the toxicity of the lewisite oxide containing trivalent arsenic, $ClCH{=}CHAsCl_2 + H_2O{=}ClCH \parallel CHAsO + 2\ HCl$. Pathologic changes in all tissues of the anterior ocular segment can be detected histologically within ten minutes after exposure, indicating the deep penetration and rapid necrotizing action of this toxic arsenical. In general, severe lewisite burns are characterized by marked conjunctival edema with petechial hemorrhages, thromboses, and ischemia; complete desquamation of the corneal epithelium with much edema, purulent infiltration, vascularization, and ulceration of the cornea; fibrinous iritis; occasionally secondary glaucoma with staphyloma of the cornea; and cataract (Fig. 88).

The rate of penetration and persistence of toxic arsenical material in the cornea and aqueous were studied by analyses for arsenic by the method of Chaney and Magnuson and by transfer of tissue juices from burned eyes to normal rabbit eyes. It was found that toxic arsenical material disappeared from the surface of the cornea within one or two minutes after the instillation of 0.1 mg. of liquid lewisite followed by closure of the lids. About 10 per cent of the original dose was demonstrated within the cornea two minutes after exposure, and detectable amounts were still present after four hours. Toxic material could be expressed mechanically from the cornea one hour after exposure but not at twenty-six hours. Toxic arsenical material was found in the aqueous two minutes after exposure and had disappeared when tested at thirty minutes.

Since lewisite disappears from the surface of the cornea and penetrates

into the aqueous within two minutes after exposure and produces irreversible changes in the tissues of the anterior ocular segment within ten minutes, a successful detoxifying agent must have the capacity to penetrate rapidly and to compete successfully for both free and combined arsenic within the tissues. For this reason, surface irrigations and neutralizing agents such as hydrogen peroxide are without value. To decontaminate the tissues of arsenic after exposure to lewisite, Peters, Stocken, and Thompson synthesized 2-3 dimercapto-propanol (CH_2SH—$CHSH$—CH_2OH) (BAL). Extensive experiments in several laboratories have been carried out to determine the optimum conditions for the use of this antidote. Although the therapeutic results have been spectacular, this substance has considerable toxicity and must therefore be used properly.

In summary, the best results have been obtained by a single instillation of 0.1 cc. of a 5 per cent solution of BAL in ethylene glycol solution or 5 per cent BAL ointment. Field tests indicated that ointment was easier for self-instillation than a solution. Such solutions and ointments instilled into human eyes produce a transitory conjunctival irritation and congestion lasting for thirty minutes to one hour, but do not produce sufficient blepharospasm to incapacitate. BAL penetrates rapidly through the cornea into the aqueous and can be demonstrated in the latter by means of the cobalt nitrate colorimeter test one minute after instillation.

To obtain excellent therapeutic results, it is essential that BAL be instilled before irreversible histologic changes have taken place. After exposure sufficient to produce a severe ocular lesion, usually with corneal perforation in the untreated eye, nothing more than a transitory conjunctival reaction and faint transitory corneal opacity results if BAL is used within two minutes (Fig. 89). When treatment is delayed for five minutes, a minor corneal opacity may persist. Treatment instituted later than thirty minutes after exposure is much less effective, but some benefit is still obtained by using BAL as late as six hours after exposure.

After exposure to a mixture of lewisite and mustard, BAL is effective in combating the lewisite component of the burn, but has no detrimental or beneficial effect on the mustard component.

Following treatment of lewisite-burned corneas with BAL, arsenic cannot be demonstrated in the cornea thirty minutes later, in contrast to the persistence of arsenic in untreated eyes.

Many BAL derivatives have been synthesized and tested on the eye, but none has a wider therapeutic range than BAL itself.

NITROGEN MUSTARD BURNS

The three nitrogen mustard compounds whose effects on the eye have been studied are HN_1 or $(ClCH_2CH_2)_2N$—C_2H_5, HN_2 or $(ClCH_2CH_2)_2NCH_3$,
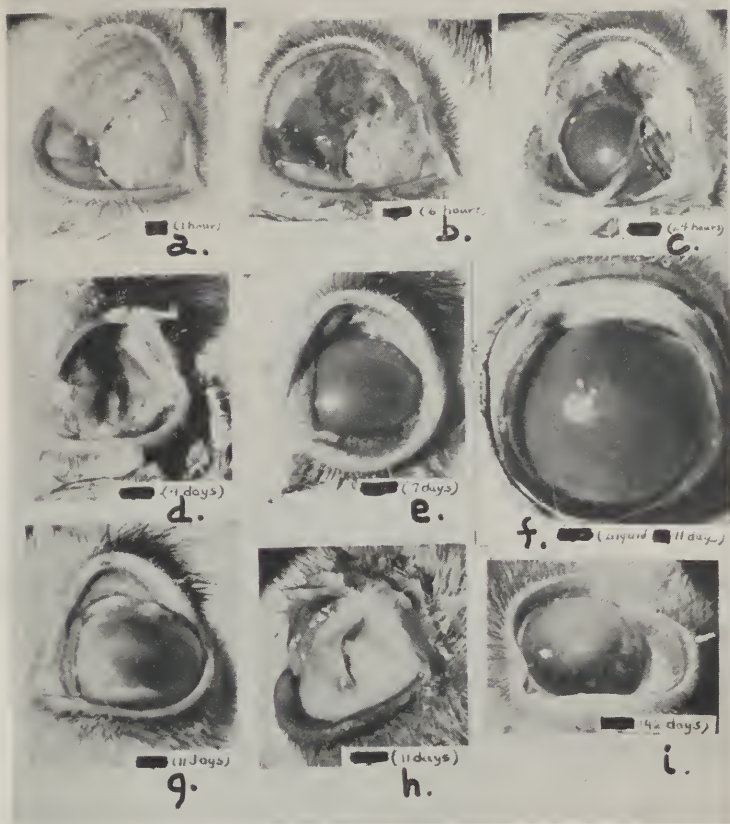
FIGURE 88. *Course of severe lewisite burns of the rabbit's eye. All eyes except that shown in f have been exposed for 30 seconds to saturated lewisite vapor at 22° C.*

FIGURE 89. LEFT: *untreated control eye 4 days after exposure to lewisite vapor.* RIGHT: *opposite eye exposed to same dose but treated 2 minutes later with 5 per cent BAL ointment.*
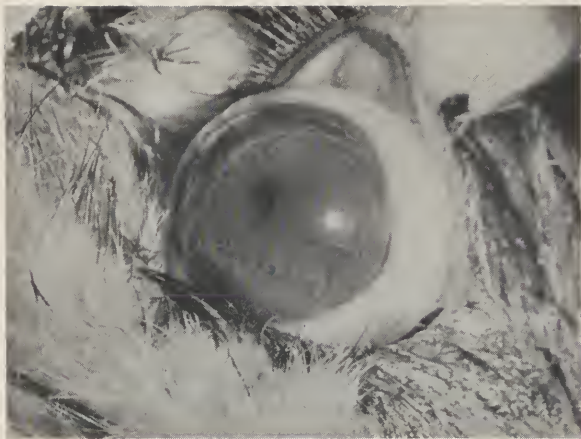
FIGURE 90. *Rabbit's eye 1 hour after exposure to HN₂, showing intense miosis.*

FIGURE 91. *Rabbit's eye 4 days after exposure to HN₂, showing moderate haziness of cornea and widely dilated pupil, with necrosis of the iris.*
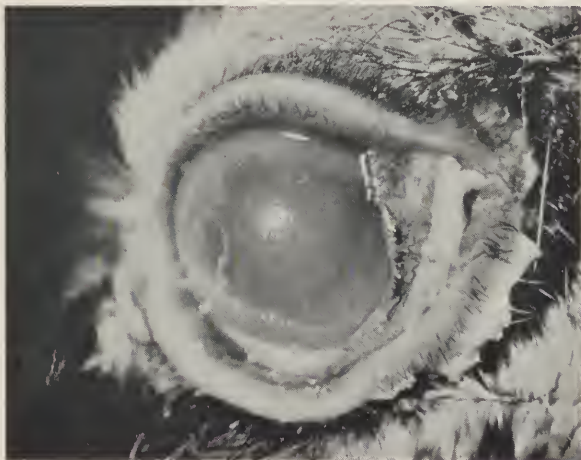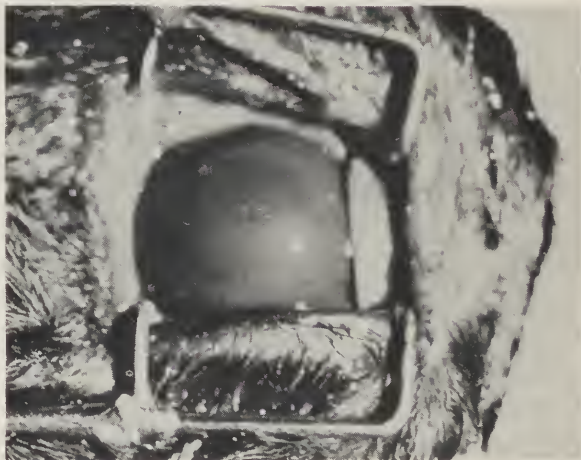
FIGURE 92. *Rabbit's eye 21 days after exposure to HN₂, showing erosion of the cornea, necrotic iris, and early cataract.*

and HN_3 or $(ClCH_2CH_2)_3N$. Their general toxicity for the eye is approximately the same as that of mustard, although one series of vapor experiments indicated that HN_3 was more toxic for both rabbit and human eyes and that HN_1 vapor was so little toxic that the animal frequently died from systemic effects without developing an ocular lesion. The major clinical and pathological differences in these three compounds lie in their degree of penetration and in the cholinergic effects produced by HN_2. Like lewisite, HN_2 penetrates the cornea rapidly, producing constriction of the pupil within five minutes, followed by serous exudation from the ciliary processes, increased intraocular tension, and later an extensive necrosis of the iris and frequently a cataract (Figs. 90, 91, and 92). HN_1 lesions, on the other hand, are mainly limited to the superficial layers of the cornea.

Efforts at decontamination have been successful two minutes after exposure to HN_2, using the following compounds listed in the order of their approximate effectiveness: BAL, NDR–620, NDR–602, and diethyl-dithiocarbamate. Diethyl-dithiocarbamate has a partial decontaminating effect after exposure to HN_1. No effective antidotes to HN_3 have been found.

EFFECTS OF ISOPROPYL FLUOROPHOSPHATE

Fluorophosphate rapidly penetrates the cornea and produces an intense miosis and spasm of the ciliary muscle because of an inactivation of cholinesterase. Although these agents were designed to disable enemy soldiers temporarily by the effects on accommodation of the eye, they are potentially useful in the treatment of glaucoma, first because the miosis persists for several days to two weeks after a single instillation, and second because they are not injurious to other parts of the eye; in fact, such massive doses can be instilled that the animal dies of systemic poisoning without developing any ocular lesion. Clinical trials with this agent in patients with glaucoma, using one drop of solution every few days, have been very promising.

MECHANISMS OF CHEMICAL INJURY TO THE CORNEA

Concurrently with the work on definitive treatment outlined above, more fundamental studies of the alterations produced by chemical injury in the normal chemical and physiological processes of the cornea were undertaken. No illusions were entertained about the chances of immediate practical success in these studies. Potential multiplicity of the receptors for mustard in the tissue and the consequent likelihood that the final tissue death is the result of a multiplicity of injuries added to the inherent difficulty of the problem. However, if one knew some of the mechanisms by which mustard injury leads to tissue death, disintegration, and repair, new therapeutic pathways might be opened.

The cornea is perhaps the ideal organ to study in the effort to bridge the gap between the primary reaction of mustard with the tissue and the subsequent development of clinically and histologically recognizable lesions. Since the cornea is avascular, it is possible in this organ to plan experiments that separate the damage and reaction of the fixed tissue components from the vascular and cellular reactions of inflammation. Also, the cornea can be maintained supravitally without difficulty, allowing one to observe the development of lesions after treatment with chemical agents in the isolated surviving tissue simultaneously with observations on its altered chemical and metabolic state. In addition, the structure of the cornea is relatively simple, so that its component tissues can be separated mechanically with ease and the effect of toxic damage to its several components individually analyzed.

In the early part of this work, a preliminary survey was made of the tolerance of the cornea for a wide variety of chemicals with known affinities for certain chemical groups, to ascertain whether toxicity could be linked to any specific combination with a tissue component. To eliminate the question of penetration through the corneal epithelium, 0.1 cc. of solutions of these substances in isotonic saline solution was injected directly into the corneal stroma. The individual symptoms produced by the injection were then graded numerically as described previously. In general, the damaging agents could be classified roughly in two groups. The first group comprised agents damaging only in large concentration; for example, acids causing indiscriminate protein precipitation, alkalis causing injury by a change in pH and loss of corneal mucoid, and dehydrating agents such as glycerol, hypertonic solutions of inorganic salts, and so forth. In the second group are the substances that are toxic in low concentrations; for example, heavy-metal ions, oxidizing and alkylating agents with a possible common point of attack in the sulfhydrils of the tissue, ketobinders such as semicarbazide and phenylhydrazine, and agents such as potassium cyanate and formalin which attack amino groups. The latter two groups are tolerated in somewhat higher concentration than the sulfhydrils. The clinical and histologic reactions elicited by these wide varieties of substances showed a monotonous similarity, indicating that these changes are only indirectly connected with the initial chemical injury and are probably the consequences of cellular death.

Certain early alterations in the cornea after exposure to mustard have been studied. Mustard, being lipoid-soluble and reactive only in aqueous media, might conceivably have its chief action at the lipoid-aqueous boundaries of the cell surfaces. The boundary between the corneal epithelium and stroma is highly impermeable to ionized substances, and this persists after exposure to mustard although the mustard itself readily penetrates this barrier. The electrical resistance of the cornea is not affected by mustard until several hours after exposure, at which time the resistance declines moderately (about 25 per cent), later decreasing to zero when the epithelium sloughs

off. It may be concluded that the lipoidal phase of the cell membrane is not disrupted as a result of the mustard reaction.

The cornea is normally in a state of turgescence and swells to give six times its normal weight when placed in water. After exposure to mustard, this phenomenon is suppressed. The possibility must be considered of a relation between this effect of mustard and a similar antiturgescent effect on red-cell ghosts and certain other cells.

After exposure to mustard, no decrease in the hexosamine content or metachromatic staining of the cornea has been found.

Experimental results given previously indicated that the rate of inhibition of corneal turgescence was directly related to the amount of both mustard and mustard-hydrolysis-intermediates that has reacted with the tissue. Because of the speed of this reaction, antidotes designed to combine with free mustard could be expected to have only a partial effect. Accordingly, bioassay studies were made on isolated tissue components to determine which substances reacted with mustard, half-hydrolyzed mustard, and divinyl sulfone to form biologically inactive complexes (for growth of rats, chicks, bacteria, and yeast cells). Mustard and its derivatives reacted with methionine and lysine in casein but not with these amino acids in corneal tissue or yeast cells. Mustard did not inactivate any of the yeast coenzymes tested; *viz.,* DPN, ATP, adenylic acid, and nicotinic acid. This indicates that mustard poisoning is not amenable to substitution therapy by these vitamin-like substances. No methods were found by which amino acids, urease, or yeast cells attacked by mustard or nitrogen mustards could be restored. Mustard sulfone, on the other hand, apparently forms a reversible combination with such substances, but the mustard sulfone itself is toxic for the eye and no completely successful decontaminating agents were found.

The attempt to discover how the metabolism of tissue is disturbed as the result of exposure to mustard may be made through two different methods of study. On the one hand, enzymes may be isolated from the tissue and exposed to mustard and the extent of their inactivation measured. It is no criticism against the important work in this field to point out that in the present state of biochemical knowledge probably only a fraction of all the cellular enzymes are as yet known, and hence that the chance of finding those crucial ones whose inactivation is responsible for particular pathologic processes is very poor. Furthermore, particular enzymes, which in homogeneous solution in vitro are found to be particularly susceptible to mustard injury, may not be readily accessible to injury in vivo. This is illustrated in one instance by the fact that the relatively large amounts of glutathione in the corneal epithelium of the cow's eye does not react with mustard in appreciable amount unless the cells are first ground with broken glass and mustard is added to the resulting emulsion. Finally, metabolic processes may be disturbed in a cell not only by direct inhibition of enzymes alkylated by

mustard but also indirectly through a disturbance of their normal interaction by structural damage within the cell, such as could result from reactions of mustard with skeletal elements of the cell and so forth.

These considerations have led to a concentration of efforts in this field on an alternate method; namely, that of discovering what metabolic pathways are inhibited by mustard injury. For these experiments, isolated surviving cows' corneas were used. The technic has been to study the utilization by the normal and poisoned tissue of various primary and intermediate metabolites injected intracorneally. The results of these studies have been reported in detail and will not be repeated here. In summary, no evidence was found that the over-all energy metabolism of tissue poisoned by mustard is seriously defective. Its consumption of oxygen, glucose, glycogen, and pyruvate is normal. There is no evidence of a general defect in its powers to phosphorylate carbohydrates. These conclusions do not, of course, imply that the available energy is utilized in a normal fashion nor that some particular channels of energy utilization may not be interfered with.

A prominent feature of corneal metabolism revealed in this work is the metabolic interaction between epithelium and stroma. The epithelium was found to have a carbohydrate metabolizing system similar to that of many other tissues involving a cyanid-sensitive oxygen acceptor, hexose phosphorylation and cleavage, and pyruvate utilization. The stroma has no measurable oxygen uptake but consumes glucose at a rate per cell twice that of the epithelium. Half of the glucose utilized can be recovered as lactate, but the tissue has no power of utilizing lactate. In the isolated cornea the lactate produced by the stroma is consumed by the epithelium, in spite of the fact that the concentration of lactate is generally higher in the epithelium than in the stroma. Conclusive proof that lactate is actively transferred from stroma to epithelium is lacking, but the utilization of the stroma lactate by the epithelium is completely inhibited by mustard poisoning. Similar results were obtained with serine.

It is to be emphasized that the interference by mustard in the intercellular metabolism is not all-embracing. Pyruvate injected into the stroma is utilized normally by the epithelium after mustard poisoning. Nor is interference with the intercellular metabolism the sole result of mustard injury. Quite obviously, the tissues even if mechanically separated from one another are susceptible to injury from mustard. We wish merely to point out that one of the aspects of mustard injury is a disturbance in the intercellular metabolism of the cornea, and that mustard furnishes a powerful tool for the study of this hitherto poorly explored field of tissue metabolism.

Loosening and sloughing of the corneal epithelium occur both in the intact animal and in the isolated incubated cornea within a few hours after exposure to mustard (Fig. 93). This phenomenon is not a consequence of the death of the epithelial cells, because small wounds in the epithelium heal

normally even when the tissue has become loosened. This pathologic process is oxidative in type, because it does not occur on anaerobic incubation; in fact, some recovery occurs during anaerobiosis, and loosening is delayed by low temperature.
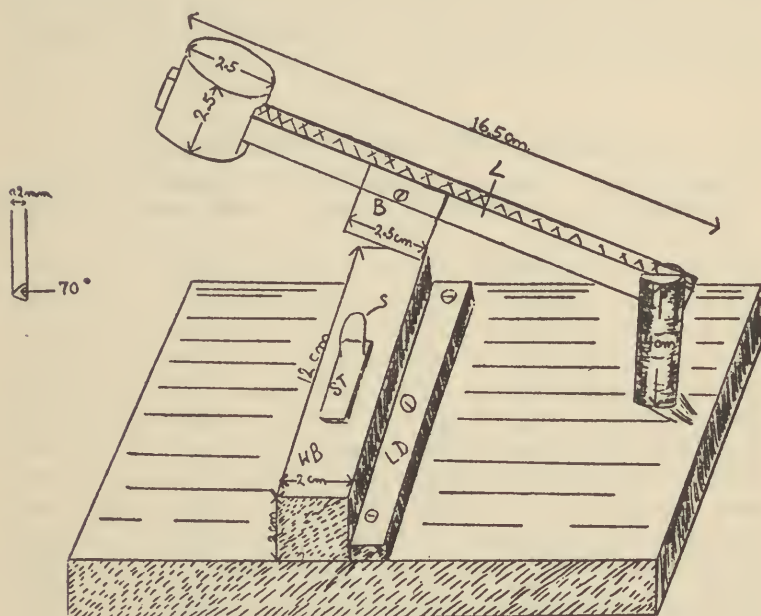
FIGURE 93. *Apparatus designed for the quantitative estimation of the degree of adhesiveness of corneal epithelium to stroma.*

The coherent surface appears to be a protein-lipoid multilayer, since it is disrupted by trypsin, butyl and amyl alcohol, and some other detergents. Energy appears necessary for the normal maintenance of cohesion because, whereas cyanide and anoxia do not cause a loosening of the tissue, iodoacetate and fluoride do cause a loosening under both aerobic and anaerobic conditions. Histamine and also freezing of the tissue with subsequent incubation also lead to a loosening of the epithelium. It is evident that this phenomenon is closely related to that of vesication in the skin.

In the search for a field of research that might diminish the enormous gap in our knowledge between biochemical studies and those of clinical and histologic pathology, the nuclear disturbances following exposure to mustard and nitrogen mustard have been studied.

Mitosis in the rat corneal epithelium is inhibited after a latent period of a few hours by a local exposure to doses of mustard or nitrogen mustard

insufficient to produce any observable metabolic or clinical effects (Table II). Cells already in mitosis at the time of the local administration show no immediate evidence of injury and complete their mitotic division normally. The healing of small wounds by migratory action of the cells is not disturbed during the period of mitotic inhibition. Such inhibition can also be seen after the intramuscular administration of an MLD_{50} dose of nitrogen mus-

TABLE II

Percentage of Mitosis in Treated versus Control Eye

Concentration of HN_2HCl	Time after Administration			Nuclear Fragments	Loosening of Epithelium
%	3 hrs.	6 hrs.	24 hrs.		
.25	43	2	0	+	+
.125	9	0	0	+	+
.06	8	8	0	+	+
.03	4	0	0	+	—
.015	15	43	0	+	0
.0075	24	20	10	+	0
.0037	32	35	6	+	0
.00175	—	38	33	0	0
.0008	—	36	52	0	0
.0004	—	53	52	0	0
.0002	—	—	84	0	0
.00005	—	—	134	0	0
.000012	—	—	147	0	0
.000003	—	—	110	0	0

tard. It therefore constitutes a part of the systemic toxicology of these agents. Recovery from the inhibition is associated with a full return to normal.

With doses of mustard or nitrogen mustard somewhat greater than the minimum required to inhibit mitosis but at threshold doses still below those required to produce clinically visible symptoms (Table II), isolated cells are seen in flat preparations of rat corneal epithelium to be undergoing nuclear fragmentation. Only a small percentage of the epithelial cells show this phenomenon even if the dose of toxic agent is greatly increased, suggesting that only cells in a particular physiological state are susceptible to this type of injury. Nuclear fragmentation from mustard appears only in the basal layers of the corneal epithelium; that is, in the layers in which mitosis also occurs. It has been shown, however, that the fragmented nuclei are not derived from cells in mitosis at the time of administration of the poison, since this lesion appears even if mitotic activity is suppressed in the cornea before the application of the mustard. These findings suggest that the cells susceptible to such injury may be those in the premitotic state. On parenteral administration of nitrogen mustard, similar nuclear fragmentation is seen

in abundance in the bone marrow, intestinal mucosa, and other rapidly growing tissues. Nuclear fragmentation may be provisionally identified as a pathologic and incomplete form of mitosis, and the use of mustard and related substances can furnish important experimental tools for the study of mitosis.

Nuclear fragmentation does not occur in the cells of the corneal stroma. Instead, the nuclei swell when water is imbibed by the cornea after damage to the corneal endothelium. After exposure to mustard, such swollen nuclei show an increased tendency to burst.

Studies of the mechanism of inflammatory reaction following mustard burns of the cornea have been limited to two negative results. Buttons of cornea removed one hour after exposure to mustard and implanted in a normal eye produced no undue inflammatory reaction in the host eye. Pressed juice of such exposed corneas did not produce a leukotaxine-like reaction when injected intracutaneously into the rabbit.

Most of the work referred to in this chapter was done under contracts issued by the Office of Scientific Research and Development to various research institutions. A list of the institutions and the individual investigators involved in this work is appended. In addition, some of the studies referred to were made by the Medical Research Division of the Chemical Warfare Service of the United States Army, Major Robert Laughlin being in charge of the Ophthalmological Division. Extensive studies in the standardization of BAL ointment were made by the Pure Food and Drug Division of the Department of Agriculture under Dr. Calvery, and co-operation in the synthesis of various substances tested for antidote value was obtained from the Chemical Department of the Experimental Station of Dupont, de Nemours Company under the direction of Dr. Lazier, and later Dr. Howk.

LIST OF INSTITUTIONS AND PERSONNEL

Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital.
    Drs. Friedenwald, Woods, Herrmann, Buschke, Hughes, Scholz, Maumenee, Snell, Guyton, and Talbot.
Howe Laboratory of Ophthalmology, Harvard University.
    Drs. Cogan, Kinsey, and Grant.
Department of Ophthalmology, University of Pennsylvania.
    Drs. Adler, Leopold, et al.
Department of Ophthalmology, Columbia University.
    Drs. Meyer, Braley; Smelzer, and Ozanics.
Department of Ophthalmology, Cornell University.
    Dr. McLean.

SUMMARY

The studies on the injury to the cornea produced by mustard were planned from the outset to include a large proportion of basic long-range investigations. The decision to undertake these projects was based on the intrinsic improbability of finding any satisfactory treatment for mustard injuries of the eyes within the sphere of the more immediate short-range type of study, and in spite of the equally great improbability that these long-range studies would come to practical fruition within the period of the war. At least the chances of success would be multiplied by increasing the number of different pathways of approach. It is no wonder, therefore, that the net result of this phase of the study has been to disclose that mustard and related compounds are useful tools in the study of a number of recondite fields of cellular and tissue physiology. In these fields more questions have been made accessible to study than have so far been answered. It seems clear that if any effective treatment is to be sought for mustard injuries these fields of study must be further explored.

The studies on BAL and on the fluorophosphates have already achieved such practical results as to establish these substances among the useful drugs as well as among the useful tools of medical research.

Part Six: Anti-Pest Agents

CHAPTER XL

THE DEVELOPMENT OF NEW INSECTICIDES

H. L. HALLER AND STANLEY J. CRISTOL

INSECT–BORNE diseases throughout the ages have been a dominant factor in determining the fate of armies and of peoples and the resulting outcomes of wars and destinies of nations. Thus, insects and related arthropods rank high in importance to the health and prosperity of nations in both peace and war. Some of the world's most dreaded diseases, such as bubonic plague, epidemic typhus, and African sleeping sickness, are exclusively insect-borne. Likewise the world's most widely debilitating malady, malaria, is chargeable to insects. To the list for which insects, mites, and ticks are responsible must be added among others Rocky Mountain spotted fever, endemic typhus, scrub typhus, yellow fever, dengue, relapsing fever, and filariasis. Nor may the part played by insects in the transmission of other diseases, such as typhoid, dysentery, and tularemia, be overlooked, nor the annoyance and impairment of efficiency of the stinging, biting, and burrowing pests — the mosquito, flea, bedbug, chigger, and the rest. Of special wartime interest is the increase in wound infections caused by the transmission of infectious micro-organisms into wounds by insect-carriers.

To the destruction wrought to man by insect-borne disease must be added the ravages of the insect against man's food supplies and his shelter. Insects carry or produce plant diseases, destroy vegetables, fruits, cereals, and sugar cane, and damage livestock and food products in storage. Devastation of forests and grasslands leads to destructive soil erosion, floods, and forest fires. This by no means completes the picture, for destructive insects feed on flowers and shrubs, gnaw holes in draperies, rugs, and clothing, undermine homes, feed on axe handles, and even interrupt communications by cutting down telephone poles or burrowing through lead cables.

But not all insects are arch enemies of man. Thousands of species are beneficial. Some insects prey on our enemy species as predators or parasites; some contribute by destroying noxious weeds; some break down dead plants or

animals, so that they are returned to the soil to serve as plant food; some work over and aerate the soil; and others furnish dyes for the fine arts, fibers for clothing, or food for beneficial wildlife, livestock, and man. In this last category the part that honeybees and other insects play in producing food often goes unrecognized. Many important food crops, including, for example, squash, apples, sweet cherries, plums, and prunes, depend largely or solely on insects to pollinate them. Furthermore, many crops essential for livestock, soil improvement, and prevention of soil erosion would but for insects be barren or produce very little seed; this is true of alfalfa, the clovers, and others.

Prior to 1941, the responsibility for research in the control of injurious insects rested almost entirely in the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture and in the experiment stations of the various states. Most of the research work at this time was concerned with agriculture, although work on such pests to man as mosquitoes, houseflies, and clothes moths was also carried out. During 1941, before the entry of this country into the war, certain medical authorities in the Army and Navy recognized the need for preparedness in efforts to combat insects among military personnel and civilians. In the fall of 1941, anticipating a need for expanded research in medical entomology, the office of the Surgeon General of the Army established contact with the Bureau of Entomology and Plant Quarantine in order to formulate plans for conducting more extensive investigations on insecticides and insect repellents for controlling mosquitoes, lice, and other insects of medical importance.

In April 1942, the Bureau expanded its facilities at the Orlando, Florida, laboratory of the Division of Insects Affecting Man and Animals with OSRD (CMR) funds. At the same time, emphasis in the Beltsville, Maryland, laboratories of the Divisions of Insecticide Investigations and Control Investigations was shifted to problems of wartime military importance.

At the commencement of the program, urgent need was evident for the control of the louse and the anopheline mosquito, transmitters of epidemic typhus and malaria, respectively. In the case of louse control, very little advance had been made over the steam sterilization of clothing used in World War I. To replace this, the use of fumigation of clothing with methyl bromide was developed, a significant advance over the earlier method. Also, the Orlando group developed a louse powder, which became known as MYL powder. It contained pyrethrum extract as the active principle against the adult louse, with N-*iso*butylundecylenamide as synergist or activator. Other ingredients included 2,4-dinitroanisole as an ovicide to prevent the louse eggs from hatching, a stabilizer to slow down decomposition of the pyrethrins, and an inert diluent. MYL powder was rapid in killing lice and provided residual action for approximately one week; it was superior to any other louse powder known at that time.

As the war went badly in the Pacific, it became apparent that our supplies of insecticidal material would be seriously affected. Possible war needs for arsenic and demands for lead threatened one of the major groups of weapons, the arsenicals. Bad weather in Kenya, as well as lack of shipping, added to our troubles by reducing the supply of pyrethrum flowers, which were especially needed in the aerosol bomb. Loss of Singapore and Malaya to the Japanese curtailed the supply of derris, the major source of rotenone. Hence, many synthetic organic compounds were tested in an effort to find suitable substitutes. It is extremely fortunate that among the many chemicals tested were included preparations containing as the active ingredient the substance now known as DDT,

$$Cl\!\!-\!\!\langle\rangle\!\!-\!\!CHCCl_3.$$
$$Cl\!\!-\!\!\langle\rangle$$

Contrary to popular reports, DDT was not smuggled out of Switzerland or Germany. In the summer of 1942, 200 pounds of insecticide spray and dust were exported from Switzerland to the United States by the Geigy Company, and samples of these materials were brought to the attention of the Bureau of Entomology and Plant Quarantine. Tests of the materials, whose active constituent was unknown to the Geigy representatives in the United States, were conducted almost simultaneously by the War Food Administration and by the investigators in Orlando. The results of tests at Orlando with DDT dusts against lice were so promising that DDT louse powder was adopted by the armed forces in 1943. Research was carried out at Orlando and at Beltsville on some of the properties of DDT, the subjects studied including analytical methods, solvents and carriers, emulsifying agents, formulations for use, specifications for purchase, and the chemical properties of the pure material.

In the meantime, entomologic testing went on apace, and it soon became apparent that DDT was more effective against a wider variety of insects than anyone had ever imagined. The effectiveness of this compound as a mosquito larvicide, against adult flies and mosquitoes (in aerosol sprays, kerosene spray, and residual deposits), and against bedbugs and the many other pests of the armed forces were all demonstrated in 1943. Finally, in the winter of 1943–1944, DDT earned its laurels by successfully aborting the Naples typhus epidemic — the first time in history that a typhus epidemic had been stopped at its peak. Until early in 1944 one company was the sole producer of DDT in this country; by that time the demand had so increased that three other companies were asked to undertake its manufacture. By the end of the war there were twelve manufacturers of DDT.

It should be realized that DDT was in effect a new compound and that very little was known of its chemical or physical properties in this country prior to 1943. This meant that everything necessary to know regarding its use as an insecticide had to be learned very rapidly and that research in all related fields had to be actively supported. Until the spring of 1944 most of the work on DDT had been conducted by the Bureau of Entomology and Plant Quarantine at Orlando and Beltsville, with pharmacologic work being carried out in the Food and Drug Administration and at the National Institute of Health. Expanding interest was reflected in an increase in the group at Beltsville and creation of new groups of investigations at three universities for work on the chemistry of DDT. Interest at that time lay in the identification of the byproducts present in technical DDT in order to establish whether they contained substances of greater insecticidal or toxicologic action than pure DDT. The Beltsville group was also charged with the responsibility of developing methods for the determination of DDT, as well as continuing a study of its analogues. Within less than six months, the composition of technical DDT had been fairly well defined, no more active ingredients than pure DDT having been found, and the university groups were transferred to the repellent program.

Groups at two state colleges and at the Edgewood laboratories of the Chemical Warfare Service studied methods for the preparation of DDT and of chloral, one of the starting materials, and their data were made available to interested manufacturers for use on a commercial scale.

Whenever a new material becomes available for large-scale use, it is necessary to provide suitable analytical methods for its detection and determination. In the case of DDT, two types of estimation are necessary — to determine first the amount of DDT in residues and deposits, and second the quality of technical DDT or of insecticidal mixtures containing DDT. Work was conducted on these problems, and reasonably satisfactory results have been obtained. Methods of estimation of DDT involving total-chlorine determinations (one half of the weight of DDT is represented by its chlorine content), or a dehydrochlorination method in which one labile chlorine atom is removed, were explored and their usefulness determined.

Perhaps the most significant contribution was the development of a specific color test for DDT. Until late in 1944, there was no specific test that could be used to detect or determine satisfactorily small quantities in the presence of other chlorine-containing compounds. At that time the Beltsville chemists developed a test that gave a blue color measurable spectrophotometrically. Other colors were obtained from compounds related to DDT, such as breakdown or metabolism products, and thus this test has been extremely useful in studying the breakdown and metabolism of DDT, as well as in determining its amount in residues and deposits. A number of other analytic methods were also developed.

For many years one of the functions of the Division of Insecticide Investigations has been the synthesis of compounds as candidate insecticides. At the time the Committee on Medical Research program was expanded, a program was started to add to the list of analogues of DDT that had been tested in the preceding year, and many new compounds were prepared. Of all the analogues tested, including several isomers of DDT, none was as effective as DDT against such a wide variety of insects, but some were successful enough for interest in them to be maintained. It was hoped that some correlation between structure and toxicity might be discovered, but no clear-cut one was noted. The idea that there might be a correlation between toxicity to insects and ease of loss of hydrogen chloride to alcoholic alkali was shown to be erroneous.

As part of the chemistry program, a study of the stability of DDT was made. It was shown that a number of substances, notably ferric chloride, iron oxide, and other iron-containing compounds and alloys, catalyze the decomposition of DDT, with consequent loss of insecticidal activity. Methods for preparing pure, stable DDT and specifications for the various grades of DDT were worked on. The properties of various dusts and diluents were studied; a principal advance lay in the development of a flowable dust containing 90 per cent of technical DDT which did not lump during shipment.

Although DDT proved to be remarkably effective in controlling lice, adult flies, adults and larvae of mosquitoes, bedbugs, some roaches, and many other pests, it was not satisfactory as a miticide or scabicide or in the control of fly-breeding places such as pits or latrines. For the latter, it was shown that ortho-dichlorobenzene and paradichlorobenzene gave good control. Tests developed formulas for control of mites (chiggers) and scabies in which benzyl benzoate was the active ingredient. The preparation of benzyl benzoate was studied. Besides the Cannizaro reaction, methods were developed for its synthesis from benzyl chloride and benzoic acid; the synthesis of benzyl chloride by chloromethylation of benzene was also developed.

As part of the regular program at Beltsville, work on the chemistry of the pyrethrins was carried out. A selective extraction procedure was developed for the preparation of pure pyrethrins, an important step because relatively pure extracts were required for the aerosol bomb. Outstanding work has also been done on the proof of structure of the pyrethrins and related compounds.

During the war the discovery of the insecticidal activity of the gamma isomer of benzene hexachloride was announced by British workers. Study of this material was conducted in the United States under OSRD(CMR) contracts, and this substance was found to be especially effective in the control of mites in their breeding places. This material must certainly be considered as one of the most promising new synthetic insecticides.

The problem of finding new insecticides is still largely an empirical one.

No satisfactory correlations between chemical structure and toxicity to insects have yet been discovered. Future work in the chemistry of insecticides must be closely allied with fundamental study of the biochemistry and physiology of insects. At the present time most of the results are available only in the measurement of insect mortality. Until the effect of various materials on biologic processes is fully understood, it will not be possible to systematize research in the chemistry of insecticides, in the sister fields of repellents and fungicides, or indeed in any of the allied chemicobiologic sciences. The value of a co-ordinating group such as the Insect Control Committee of the Office of Scientific Research and Development in such an undertaking is obvious. Research is expensive. It is often difficult or impossible to obtain substantial budgets for so-called "academic" research in fundamental science. Provision of research funds to responsible university, government, and foundation researchers for the study of the biochemistry and organic chemistry of insects and insecticides should help in the solution of important problems. Other functions of the group lie in the collection and dissemination of information in related fields and in sponsorship of meetings where interested workers may discuss their results and exchange ideas and information.

THE ACTION OF DDT ON INVERTEBRATES

J. FRANKLIN YEAGER

After samples of DDT, originally reported by Swiss scientists to be an unusually effective insecticide, had been received in this country, the Swiss claims were soon confirmed by the results of numerous laboratory and field tests. The new insecticide showed promise of widespread application, and as a consequence several general questions arose in connection with it. One of these was the problem of its mode of action in insects and other invertebrates. The problem was investigated by scientists working in various localities and institutions but co-operating through the agency of the Office of Scientific Research and Development. How they approached the problem, and with what results, will be described.

The most obvious symptoms in an insect poisoned with DDT are twitches, tremors, spasmodic convulsions, unco-ordinated movements of the appendages and body, loss of equilibrium, and prostration. These suggest that DDT may have an effect on the insect's nervous system; in fact, evidence for such action had already been reported by the Swiss investigators. It was reasonable, therefore, that the problem of the mode of action of DDT should be attacked vigorously from this standpoint.

Efforts were made to demonstrate that these symptoms, facetiously called "the DDT's," were the result of a direct action of the poison on the central nervous system of an insect, but thus far little or no positive evidence of such an action has been obtained. Application of DDT, for example, to the isolated central nervous system of the roach failed to influence the rate of the spontaneous electrical outbursts characteristic of the preparation. The results of other observations and experiments suggested to investigators that although DDT seemed to have no direct action on the central nervous system, an intact reflex mechanism must be involved in the production of the muscular movements during at least the earlier stages of poisoning by low or moderate doses of DDT and, further, that DDT must in some way affect the sensory mechanism of the insect. With the use of electrophysiological methods, results were obtained leading to the conclusion that when an insect is poisoned with low or moderate doses of DDT, the rapid onset and continuance of the symptoms of poisoning are brought about through an action of the poison on certain sensory receptors, with a consequent marked, in-

discriminate, and disorganizing bombardment of the motor neurons in the central nervous system.

A number of investigators obtained indirect evidence that when given in sufficiently high concentrations DDT could also affect peripheral motor-nerve fibers in insects, causing a repetitive discharge of motor impulses. Direct proof that DDT has an action on some of these nerve fibers was obtained in crustaceans. It was found that DDT if applied to an exposed nerve in a leg severed from a crab caused a repetitive discharge of impulses in response to a single, brief electrical stimulus, instead of a single discharge as would otherwise be expected. With higher concentrations, the multiple discharges were longer, and eventually spontaneous discharges occurred. Similar experiments on roaches indicated that analogous results were to be expected with insect material.

These experiments, considered together, offer very strong evidence that in the insects and crustaceans DDT acts on certain sense cells and peripheral motor-nerve fibers. That crustacean peripheral nerve fibers are affected by the poison has also been proved.

Other investigations were directed toward finding out whether DDT caused detailed cytologic changes in the insect nervous system. The insect nerve fiber contains a central axis cylinder and a surrounding lipid sheath, comparable to that found in young vertebrates prior to the appearance of visible medullation. Birefringence studies, in which polarized light was used, failed to indicate injury by DDT either to the axoplasm or to the lipid sheath, although certain other insecticides or repellents affected the one or the other or both. DDT, as well as a number of other poisons, seemed to cause no specific histopathologic changes in the nervous system prior to a loss of responsiveness to stimulation. Results from further experiments indicated that DDT symptoms in other arthropods were lessened or abolished by calcium ions, that in lower toxic concentrations DDT exhibited a negative temperature coefficient (characteristic of adsorption phenomena), and that DDT was readily and reversibly adsorbed by certain surfaces. All these results are of particular interest from the viewpoint of the proposal that DDT poisoning may involve an interference by adsorbed DDT molecules with the normal interaction between calcium ions and the nerve membrane. Interesting also in this connection is the fact that the DDT symptoms were decreased or abolished by anesthetics or narcotics.

Although sites of action of DDT in the arthropod nervous system have been indicated, numerous attempts to demonstrate the biochemical mechanism involved in DDT poisoning have yielded a less clear-cut picture. Indeed, this problem is beset with difficulties, partly because of the small amounts of material that must be used. In view of the obvious neuromotor disturbances evoked during DDT poisoning, studies were made of the acetylcholine-cholinesterase system. In vivo studies showed that when a

roach poisoned with DDT reached the stage of prostration, the amount of free acetylcholine in its central nervous system was greatly increased. This increase was found to take place in the bundles of nerve fibers between the ganglia rather than in the ganglia themselves, which contain nerve cell bodies. It appears that the rise in free acetylcholine did not result from an inhibition of cholinesterase by the DDT. Attempts to demonstrate a similar increase in vitro have yielded negative results.

These experiments indicated that, in some way at present unknown, DDT poisoning involves a change in acetylcholine. A hypothesis, in agreement with some experimental results, was proposed that DDT and acetylcholine may compete for lipoprotein, with the consequent liberation of lipoprotein-bound acetylcholine. The birefringence studies mentioned earlier suggest that a competition of this sort taking place in the DDT-poisoned insect would probably not alter the birefringence characteristics of the lipid nerve sheath.

The rise of acetylcholine associated with prostration was of interest with regard to a report that DDT-poisoned flies exhibited a marked increase in oxygen uptake during a less active stage of poisoning, also associated with prostration. Seeking a relationship of oxygen intake to muscular activity and associated changes, other investigators found that DDT could increase respiration in other insects and, further, that the increase could be abolished by a narcotic. These experiments led to the view that the observed rise in respiration might be attributed to increased muscular activity in the poisoned insects. It is apparent that more extended research is required in order to ascertain the various factors influencing respiratory metabolism under these conditions.

Other metabolic changes in insects undergoing DDT poisoning were sought; for example, changes in body weight, water content, ether-extractable material, nonprotein nitrogen, glycogen, glucose, and nonfermentable reducing substances. Of particular interest were the marked decreases in glycogen and glucose associated with the violent muscular activity during poisoning.

Exploratory determinations were made of the effects on insects of a number of drugs and compounds other than DDT. Among the results obtained was evidence that DDT symptoms could be alleviated or abolished by atropine, barbiturates, and certain anesthetics; the activity was abolished also by nicotine, in concentrations sufficient to cause paralysis. Eserine produced symptoms in injected roaches suggestive of those induced by DDT and hastened the death of insects poisoned with it. Chloral hydrate injected into roaches tended to have a depressant effect, but sometimes caused tremors to take place in the terminal stage of poisoning shortly before the insects died, usually not earlier. The injection of large doses of acetyl-β-methylcholine into the roach, the nervous system of which was found to contain an esterase

active against this compound, did not affect that insect, whereas the injection of small doses of carbaminoylcholine, for which the insect possesses no known esterase, caused immediate symptoms and early death. Whatever the mechanisms of these and other reported effects, whether simple or complex, their significance with respect to the mode of action of DDT may be revealed in the course of future research.

A series of tests on a wide variety of aquatic organisms showed a definite relation between the possession by an organism of a chitinous integument or body covering and the susceptibility of the organism to the toxic action of DDT applied externally. It was further shown that insect cuticle would adsorb DDT from solution and that at lower concentrations DDT toxicity was associated with a negative temperature coefficient, suggestive of an adsorption process, although at higher concentrations the coefficient was positive. These results led to the hypothesis that the penetration of DDT into the body of an animal having a chitinous integument or outer membrane may be facilitated by the poisons becoming adsorbed by the chitin and selectively concentrated in the integument. The change from a negative temperature coefficient at low concentrations to a positive coefficient at high concentrations suggested that the adsorption mechanism in the cuticle is not identical with the mechanism of the lethal action of DDT in the organism.

DDT was found to penetrate through different areas of the insect integument and to become distributed variously to the different tissues. Investigators made use of a radioactive bromine analogue of DDT and employed radioactivity as an indication of the presence of this compound in insect tissues. The penetration into the roach of the analogue applied externally was more rapid from some regions of the integument than from others. After injection, when marked tremors were occurring, radioactive material was evident in some tissues but not in the fat body or excretory tubes. Although a fine localization of radioactive material in histologic structures was not possible, it would seem that, in the early stage of poisoning, excretion of this poison must have been slight or absent and that accumulation in the fat tissue must not have taken place rapidly.

These researches, considered together, substantiate the opinion that the violent tremors and contractions of a DDT-poisoned insect may result from the action of DDT at sites in sensory and motor portions of the nervous system. Although positive evidence of a direct action on the central nervous system is lacking, this system is involved in the reflex action associated with those symptoms. The information that has been obtained is not yet sufficient to show whether or not the death of a poisoned insect results directly from the action of the poison at nerve sites. The suggestion was made that the organism dies from exhaustion, after having used up its available carbohydrate or other food reserves in muscular contractions during poisoning. On the other hand, it was demonstrated that a number of compounds evoked

DDT-like tremors and contractions when injected into insects (roaches) and crustaceans. The roaches recovered from the neuromotor symptoms caused by some compounds, and yet died subsequently from the effects of the poison. The life of crustaceans poisoned with DDT was prolonged and recovery was facilitated through the use of mild anesthetics that reduced the violent muscular activity and prevented exhaustion. It is apparent that the sites and mechanism of the lethal action of DDT require further investigation.

In addition to the physiological hypotheses mentioned, two general chemical hypotheses relative to the toxic action of DDT have been proposed. One, put forth by the Swiss investigators and developed in connection with their researches on DDT and other compounds, lays emphasis on molecular configuration, lipoid solubility, and combination with certain tissue components; for example, a sterol. The other, based on work in England, stresses lipoid solubility and the splitting off of hydrochloric acid at the sites of action in the tissues. At the present time, neither hypothesis seems to aid very much in the detailed interpretation of the results of these experiments on the mode of action of DDT in invertebrates. It seems that the formulation of a generally acceptable theory of the lethal action of DDT must await the acquisition of more experimental data. Obviously, it is necessary to continue research along the promising lines of attack that have been opened up, as well as in directions that will appear in the course of future investigations.

THE TOXICOLOGY AND MECHANISM OF ACTION OF DDT IN MAMMALS

RICHARD A. ORMSBEE

AFTER DDT was shown to be a powerful insecticide, intensive investigation of its toxicity to man became imperative; this information was of vital importance for its safe and efficient use as an insecticide. Consequently, representatives of the National Institute of Health, the Food and Drug Administration, the Kettering Laboratory of Applied Physiology, and the National Research Council met with representatives of the armed services and mapped a program to provide the necessary information. As this program proceeded it was accompanied by close and effective liaison with the Bureau of Entomology and Plant Quarantine. Progress in the investigations was reviewed at a series of meetings.

It was realized that DDT might be incorporated in dusts, sprays, mists, and aerosols. The investigations therefore involved the effects of inhalation, ingestion, skin sensitization, absorption through the skin, and the dangers associated with the commercial production of DDT.

By 1944, when the Insect Control Committee of the Office of Scientific Research and Development was formed, work had progressed to the point where it was recognized that certain DDT formulations could be used extensively and safely by the armed forces. At this time the available information was reviewed to ascertain further desirable investigations. Stress was laid on studies of the mechanism of action of DDT in insects and mammals, additional toxicity studies in man and animals, absorption of DDT through the skin, neurologic effects, the effect of diet and the intermediary metabolism of DDT, the development of resistance to the compound, and the development of therapy for DDT poisoning. The expanded research program required collaboration from additional institutions. Correlated investigations of the toxicity of solvents and emulsifying agents needed for the dispersal of DDT were also undertaken, as well as the study of analogues of DDT of interest as possible new insecticides. Other new insecticides, including hexachlorocyclohexane (666) and pyrethrins, were investigated. Work was also encouraged on the pharmacology and mechanism of action of rodenticides, including sodium fluoroacetate (1080) and alpha-naphthylthiourea (ANTU). Another project involved extensive studies on the toxicity and irritancy of

compounds designated as candidate insect repellents. This was co-ordinated with concurrent testing of the same compounds as insect repellents.

It was shown that the symptoms of DDT poisoning in mammals are quite similar to those in insects. They involve a primary tremor, which becomes coarser, and spasmodic muscular contractions, followed and interspersed by tonic and clonic convulsions, with the animal dying in a depressed state. In larger animals such symptoms may be abruptly terminated by fatal ventricular fibrillation.

DDT acts primarily on the central nervous system of mammals, in contradistinction to its peripheral locus of action in insects. The principal effects center in the motor cortex and the cerebellum. In common with many other chlorinated hydrocarbons, DDT sensitizes the myocardium to epinephrine, with consequent fatal ventricular fibrillation. This is particularly apparent in larger mammals such as monkeys and dogs.

Metabolic studies with DDT showed that p,p'-dichlorodiphenyl acetic acid (DDA) is excreted by the kidneys. Correspondingly, the in vitro degradation of DDT in hot alkaline solution produces the same end product; it is nontoxic.

Studies on the distribution of DDT in the body tissue of animals revealed that high concentrations are built up in fatty tissues of the body, particularly in neutral fat depots. An animal receiving a series of subacute doses of DDT continues to excrete DDA in the urine for some time after termination of administration of DDT. This is interpreted as associated with the slow release of unchanged DDT stored in the fat depots in the body, which may therefore serve as safety reservoirs for DDT and thus prevent the appearance of acute symptoms. Such a deduction is strengthened by the observation that animals with a great deal of body fat are less susceptible to acute DDT poisoning than are animals lacking such deposits. These fat depots may, on the other hand, prolong symptoms of poisoning in animals after ingestion of DDT has ceased, since it may be slowly released into the body in sufficient quantities to cause such symptoms.

Biochemical and pharmacologic investigations indicate no direct involvement of any enzyme system and no significant changes in nonprotein nitrogen, blood phosphorus, glucose, serum bilirubin, or whole-blood lactic acid when the animals are kept quiet. The lactic acid may rise to high levels during periods of tremor and convulsion, and for the same reason the glycogen stores may be severely depleted. Hepatic and kidney clearance tests have indicated little dysfunction. Although utilization of pyruvate may be subnormal, this is thought to be due to functional liver damage.

The metabolism of animals may be significantly increased during DDT poisoning. This is reflected by increases in the oxygen consumption of the whole animal and of tissue slices from poisoned animals.

The morphologic picture in poisoned animals is one of slowly progressive

liver damage, slight muscle necrosis and involvement of the central nervous system, with changes in the anterior horn cells, some vacuolization around larger nerve cells in cord and cerebral motor nuclei, and, in some cases, significant lesions in the roof and dentate nuclei of the cerebellum.

The lethal dose of DDT varies depending on the vehicle and mode of administration. Orally, depending on the animal, the LD_{50} dose dissolved in oil ranges from 150 mg./kg. in rats to over 400 mg./kg. in mice. Administered intravenously as an emulsion, the LD_{50} dose for most animals, including monkeys, lies between 30 and 75 mg./kg.

No specific therapy for DDT poisoning was found. Effective symptomatic therapy consists of the use of central depressants, with sodium phenobarbital the agent of choice. In dogs, limited relief from symptoms may be obtained by the use of calcium gluconate. In cases of chronic poisoning where liver damage is likely, a diet low in fat and high in protein, carbohydrate, and calcium has been recommended.

The extensive use of DDT as an insecticide in this country and in the theaters of operation of the armed forces has not resulted so far as is known in a single example of poisoning *per se*. Cases of poisoning with formulations containing DDT have been shown in every case to be due to the solvent or to other compounds in the preparation. A number of effective DDT formulations and solvents have now been exhaustively investigated, with the result that there are available for use a number of such preparations, which are safe and are recommended by the Department of Agriculture as effective insecticides. These include dusts, solutions, and emulsions.

Information on the effects of the ingestion of small amounts of DDT over long periods of time is still incomplete. This information is important since it involves the use of DDT on crops and its effect on farm animals, including milk cows.

Under experimental conditions toxic amounts of DDT may be excreted in the milk of laboratory animals. Similar excretion in the milk of cows has likewise been demonstrated. Other problems involve the long-continued exposure of human beings to small amounts of DDT. This problem will require careful observations over a period of years for elucidation.

The Insect Control Committee has assisted in this program by co-ordinating the work of the Office of Scientific Research and Development contractors with that of other co-operating agencies, by issuing current reviews of the data, and by holding frequent meetings for the discussion of results and problems. The transfer of the Insect Control Committee from the Office of Scientific Research and Development to the National Academy of Sciences in the summer of 1945 caused no essential change in these activities. With the termination of hostilities, the Insect Control Committee turned from its wartime duties and devoted itself to the peacetime research needs of the United States in the control of insects and rodents.

In the fall of 1946, the Insect Control Committee was transformed into the Chemical-Biological Coordination Center of the National Research Council. The activities of this organization are devoted to the codification and correlation of data relating chemical structure to biologic action.

THE DISPERSAL OF INSECTICIDES

HARRIET A. GEER, RANDALL LATTA, AND ARTHUR W. LINDQUIST

As A RESULT of the world-wide distribution of military personnel during the war, insect control was required under a variety of conditions. For example, control was necessary in actual combat zones, in jungle or other uninhabitable areas where troops were stationed for long periods of time, and in camps in this country. Adaptation to the different conditions encountered in practical control is accomplished by variations in the method of dissemination. In the dispersal of any insecticide several factors are important in determining the method used; namely, the size and accessibility of the area to be treated, the nature of the terrain, the type of vegetation, and, in military operations, the proximity of the enemy. Until recently little work had been done on particle size in relation to the dispersal of insecticides.

Investigations of dispersal methods proceeded hand in hand with the development of equipment designed to fulfill the requirements indicated by small-scale investigations. By practical tests in the field such equipment was examined to determine its actual usefulness in insect control. In some instances the methods for dispersal were developed in the field in response to definite needs.

The discovery of DDT as an insecticide opened a new era in insect control. The small quantities of DDT required for insect kill and the extensive residual properties exhibited by this substance made it necessary to revise dispersal equipment. Aircraft dispersal became an important means of controlling insects in large areas. Formulations for satisfactory DDT dispersal were also called for.

FORMULATIONS

In early experiments with DDT, dust as a carrier was not highly successful for large-scale dispersal. When 10 per cent DDT dust was used as a larvicide, no saving in the amount of labor resulted. Although recently a powder containing as much as 90 per cent DDT has been developed, it was not originally possible to obtain satisfactory micronization of the DDT without the presence of a large amount of an inert substance. This required the transportation of large quantities of material, which is undesirable when

remote areas are to be treated. In the early distribution of DDT a 5 per cent DDT-oil solution (generally kerosene, diesel, or fuel oil) was widely used. Although it was possible to prepare this solution in the field, it was considered desirable to prepare a concentrate that could be shipped to the war theaters. The Department of Agriculture developed such a concentrate for the preparation of emulsions at the site of application. Somewhat stringent criteria were set up for this concentrate, with the purpose in view of developing a foolproof concentrate that could be used with soft, hard, or sea water. A concentrate containing 20 per cent DDT, 20 per cent Triton X–100, and 60 per cent xylene was originally recommended for the use of the armed services. Further investigations showed that a concentrate containing 25 per cent DDT, 10 per cent Triton X–100, and 65 per cent xylene formed emulsions of sufficient stability. Whereas from the viewpoint of stability this is a satisfactory emulsion concentrate, the flash point of xylene is low, which offers a hazard in its use. Research was directed toward finding a substitute solvent with a higher flash point and with higher solvent properties for DDT at low temperatures. Several solvents were investigated, and the Department of Agriculture recommended three formulations containing 25 per cent DDT and 15 per cent Triton X–100, with PD–544C, alone or in conjunction with cyclohexanone or isophorone, as a solvent. Research has been carried out to find a substitute emulsifier for Triton X–100, which is a relatively expensive substance. Duponol OS, Alkanol WXN, Ammonyx OO, and equal mixtures of Tween 20 and Span 20 have all shown promise as emulsifier substitutes. Actually in practical control, when sea water is not used, as little as 1 to 2 per cent Triton X–100 may be used in the xylene formula.

In the search for a substance having high solvent properties for DDT, considerable investigation has been made of the polymethylated naphthalenes. These are marketed by a number of companies under such trade names as Velsicol AR–50, AR–60, AR–70, and NR–70, Koppers K–327, Koppers Kolineum, Sun Aro-Sol (151–B), APS–202, and PD–544C. These solvents will dissolve over 30 per cent DDT and may be used as auxiliary solvents in conjunction with diesel or fuel oil. The toxicity of seven of these solvents has been extensively investigated by the National Institute of Health and the Food and Drug Administration. Because of their skin-photosensitizing properties, Koppers Kolineum, Velsicol NR–70, and Sun Aro-Sol (151–B) were not recommended as DDT solvents when contamination of the skin was likely to occur. The other four solvents tested — APS–202, Velsicol AR–60, Koppers K–327, and PD–544C — were considered safe for use as DDT solvents when precautions were taken to prevent excessive inhalation exposure of human beings. However, all these solvents were less irritating to the skin than kerosene.

Realization that dispersal of DDT as a water suspension would be highly desirable led to investigation of the preparation of a dispersible powder. Al-

though this product has not had an extensive field trial, such preparations have been developed and show promise as a means of dispersing DDT.

STUDY OF PARTICLE SIZE

The optimum particle size for distribution of insecticides had been investigated to some extent prior to the discovery of DDT. To study this problem more thoroughly both theoretical and experimental investigations were undertaken by Division Ten of the National Defense Research Committee. In experimental tests carried out in co-operation with the Bureau of Entomology and Plant Quarantine under contract with the Committee on Medical Research, Division Five, the toxicity of a homogeneous DDT aerosol to adult *Aedes aegypti* females in a static chamber was found to increase 250-fold when the diameter of particles was increased from 0.4 μ, that of screening smoke, to 16 μ. Under these conditions the toxicity varied up to a limiting particle size with the square of the diameter of the droplets. Optimum kill was noted when an aerosol of 10 μ in diameter was employed.

Theoretical calculations based on the impactibility of different-sized drops, the estimated dose required to kill a mosquito, and statistical considerations predicted an optimum drop size of the same order of magnitude, although a minimum dosage was indicated at a somewhat higher value for drop diameter and corresponded approximately to the size at which one drop contained a lethal dose of DDT.

To study the behavior of aerosols under conditions more closely approximating those in the field, wind-tunnel studies with *Aedes aegypti* as the test insect were undertaken at the Beltsville laboratory in Maryland. Mortality was determined with particles varying continuously from 1 to 20 μ in diameter and at wind velocities of 2, 4, 8, and 16 m.p.h. As a result of these studies it was shown that under conditions where only a small fraction of particles deposit on insects in their path (small particles, low velocity), the amount of aerosol required is inversely proportional to the product of the diameter (D) squared and the wind velocity (v). This increase in effectiveness with increase in particle size and velocity continues until a point is reached where almost all particles are deposited on an insect in their path. Total deposition is approached when D^2v exceeds 1000 (D, microns; v, m.p.h.), although there are only slight differences in dosage for values of D^2v greater than 300. These experiments indicate that the optimum particle size depends on the combination of conditions at the time of application. It was shown that increasing the drop size beyond 20 μ in diameter did not increase the deposition on the insect.

In practical control, factors such as the meteorologic conditions, the type of terrain, and the method of dispersal will be deciding factors in the drop size indicated for use.

GROUND DISPERSAL

In the control of adult mosquitoes, kill may be accomplished by contact of the insect either with the air-borne insecticide or with a residual deposit of the insecticide. Contact of the flying insect with the insecticide brings about control more rapidly, but a residual deposit of DDT in buildings may remain effective for mosquito control for periods of three or four months. To obtain kill of the insect in flight, the insecticide should be dispersed in a form that will remain air-borne for a considerable time. This is achieved by the use of aerosols. On the other hand, to obtain a satisfactory residual deposit, larger particles, which will give a uniform coverage of the treated surface, are indicated.

Atomized Sprays or Aerosols

Preliminary tests with screening-smoke generators for the dispersal of DDT indicated that the use of an aerosol would be an effective method of controlling insects over a large area. These tests, conducted by Division 10 of the National Defense Research Committee late in 1943, also showed that the particle size of screening smoke is not the optimum one for insecticidal dispersal. Thereupon, Division Ten undertook the development of a generator that would emit particles larger than those of screening smoke. As a result of these efforts, an insecticidal generator known as the Hochberg-LaMer generator was developed. This equipment, which uses an emulsion, operates on an entirely different principle from that of the coil-type screening-smoke generators. The water, but not the oil, is vaporized at temperatures of 300 to 500° F., and nozzle pressures are 80 to 120 psi. Factors influencing particle size, which may be varied from a smoke to a coarse aerosol, are temperature, pressure, emulsion formulation, and nozzle characteristics. Although the aerosol is not completely homogeneous, a large portion is present in any narrow desired size range, depending on the conditions of operation. The Besler M–2 (Army) and No. 374 (Navy) smoke generators were remodeled in accordance with the Hochberg-LaMer principles, and field tests were carried out in this country and in the war theaters. These showed the generator to be effective in controlling insects in wooded areas 300 to 400 feet downwind and in the open as far as 5000 feet downwind with a dosage of 0.25 lb./acre. The converted Army generator, designated as the E–12, is now under investigation by the Chemical Warfare Service, and further modifications are in progress.

The Department of Agriculture found that either hand-operated or power-driven paint-type sprayers gave excellent control with concentrated solutions of DDT. It was shown that 1 ml. of a 20 per cent solution was as effective as 20 ml. of a 1 per cent solution, provided it was atomized to particles of 5 to

15 μ in diameter. Under jungle conditions, as little as 40 ml. of a 20 per cent solution per acre (0.017 lb./acre) gave 90 per cent reduction of mosquitoes within one hour after spraying.

At the Munitions Development Laboratory of the University of Illinois, a simple device for attachment to the exhaust of a jeep was designed. This consists of a venturi atomizer that employs the high-velocity exhaust gases from an internal-combustion engine to break up the insecticidal solution as it is injected into the venturi throat. This device has been successfully adapted to the cargo carrier M–29C ("Weasel") and shows promise of being adaptable to civilian motor vehicles. By adjustment of the rate of flow of the solution, either an aerosol or a coarse spray may be obtained. Preliminary field tests in Florida and Hawaii have indicated that this is a satisfactory method of controlling insects in small areas.

Prior to the discovery of DDT, the Department of Agriculture had developed a pyrethrum aerosol bomb for control of insects in confined spaces. Freon-12 (dichlorodifluoromethane) is incorporated with the insecticide and acts as a propellent when the nozzle is opened. Atomization is accomplished by the shearing forces exerted by the escaping gas on the insecticide solution. Particle-size determinations have shown that this device emits particles 5
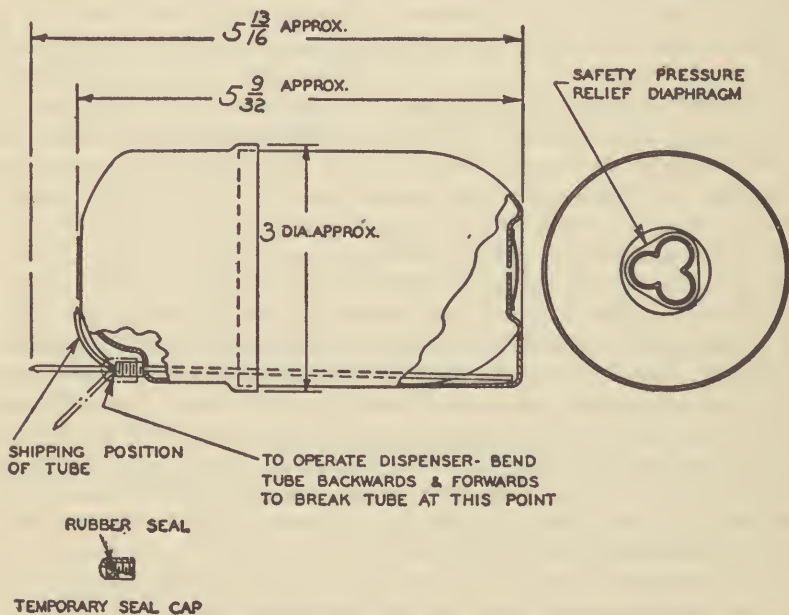
FIGURE 94. *Diagram showing the construction and operation of the aerosol bomb.*

to 20 μ in diameter. During the war DDT, in addition to pyrethrum, was incorporated as an active ingredient. Because of the low solubility of DDT in Freon-12, an auxiliary solvent is required to keep it in solution. Toxicity tests on the formulations developed were carried out by the National Institute of Health. These bombs (Fig. 94) were used by the Army in both forward and rear areas. A pocket sprayer using a 20 per cent DDT concentrate was designed. This sprayer is less cumbersome than the aerosol bomb and is equally effective from the point of view of insect kill. Flit-gun sprayers are also effective in space spraying. In the early part of the war considerable difficulty was experienced because of unsatisfactory equipment. Gaskets and other rubber fittings were not oil-resistant, and priorities had not been assigned for the metals used in sprayers. As a result, a large portion of the sprayers were quite unsatisfactory. These difficulties have been eliminated, and a satisfactory continuous hand sprayer of 2-quart capacity is now a Quartermaster issue.

The British had shown considerable interest in the development of a DDT grenade. Such a device was developed by the Chemical Warfare Service at Edgewood Arsenal and tested for its entomologic performance by the Department of Agriculture. Whether or not this will prove to be an effective method of dispersal is questionable, since some decomposition of DDT occurs at the high temperatures used and the particle size is less than that believed optimum for the most effective kill.

Residual Treatment of Buildings and Other Areas

Residual deposits of 100 to 200 mg. of DDT per square foot on the interior surfaces of buildings may remain toxic to mosquitoes for several months. For the control of adult mosquitoes and flies the ordinary 3- to 4-gallon garden-type sprayers were found to emit the spray too rapidly for use with DDT. By changing the size of the orifice to 56 to 60 wire gauge and equipping the sprayer with oil-resistant hose and washers, satisfactory dispersal could be carried out. For the application of a residual spray a nozzle that delivers a fan-type spray has proved more satisfactory than the disk nozzle, which produces a cylindrical pattern. This nozzle may be attached to either a hand or a power sprayer. The Corps of Engineers designed a cylindrical knapsack sprayer of 3-gallon capacity, which contains no rubber fittings or gaskets and is completely satisfactory for application of residual sprays.

Power sprayers of the agricultural type then in use delivered the spray at too rapid a rate for economical dispersal of DDT. Husman and Longcoy constructed a small, light power unit, which may be transported by hand if necessary or mounted on a small truck or hand cart. This unit gives a constant pressure in the range of 10 to 60 psi and a maximum delivery rate

from a 60-gauge orifice of 1 qt./min. By a simple change of nozzle the emitted spray may be varied from a coarse spray to a fine mist.

LARVICIDING

As was true in the control of adult insects, the existing equipment delivered the spray too rapidly for economical larviciding with DDT. Reduction of the orifice of the ordinary 3- to 4-gallon cylindrical sprayers to 56 to 60 wire gauge allowed their use for larviciding operations with a 1 per cent DDT-oil solution. Hand or power paint-type sprayers have proved useful for larviciding. Because of the small droplets the spray drifts from 200 to 600 feet downwind. The Hochberg-LaMer generator, using a dosage of 0.1 lb./acre, has been employed successfully in the treatment of large inaccessible areas. The jeep exhaust generator shows promise of being a satisfactory method for the dispersal of DDT as a larvicide. For treating small breeding places such as road ruts the 2-quart continuous-pressure sprayer is very adaptable. DDT may be used as a dust, but no saving in labor over that required for dispensing Paris green dust is obtained.

AIRCRAFT DISPERSAL

Prior to the introduction of DDT, no insecticide that could be economically produced in large quantities had been sufficiently toxic to warrant its distribution as an aircraft spray. Preliminary tests were carried out in 1943 over rice fields near Stuttgart, Arkansas, with 5 and 10 per cent DDT dust distributed from a Stearman biplane equipped with a commercial dusting unit. Although the results were promising against both anopheline larvae and adults, a dust was not considered the most satisfactory method of dispersing DDT.

SPRAY TANKS

Chemical-warfare spray tanks were available in the war theaters for DDT dispersal from fast military aircraft. Several investigators worked on modifications of this equipment to produce sprays with small droplets, and by modification of the nozzle and restriction of the orifice an improvement in the spray pattern was made. In cross winds of 3 to 15 m.p.h. swath widths of 100 to 400 yards were secured. The pay load with such tanks was too small for economical dispersal, but with the use of auxiliary fuel tanks large loads of insecticide could be accommodated. When such tanks are employed the B–25 plane has a capacity of 550 gallons and the C–47 800 gallons. In a co-operative project between the Army Air Forces Board at Wright Field and Division Ten of the National Defense Research Committee, at the University of Illinois, a simple vertical discharge pipe was designed. This pipe is cut off at a 45-degree angle, emission occurs by gravity flow, and atomization

results from the air turbulence at the emission outlet. In field tests carried out in Panama over dense tropical jungle with dosages of 0.3 and 0.6 lb. DDT/acre, nearly 100 per cent control of anopheline larvae and adults was obtained. In view of the simplicity of this device it was adopted as standard dispersal equipment for DDT by the Army Air Forces. For use on fast military aircraft this is very satisfactory, but the air stream from small planes is insufficient to give satisfactory breakup. For civilian use this equipment will probably have little value except for control work in extremely large areas.

Specially Designed Spray Equipment

A unit for spraying DDT liquid from a Cub airplane was designed and built by Husman and Longcoy in 1943. A wind-driven gear pump drew the liquid from the 25-gallon spray tank installed in the forward cockpit and forced the spray under about 70 psi pressure into a series of nozzles located in a venturi under the fuselage of the plane. These nozzles ejected the spray against a round plate, which produced a better breakup of the liquid. This equipment, using a 5 per cent DDT solution or emulsion, was tested against adult mosquitoes over dense mangrove jungle on the Florida Keys and later in the Pacific Theater. Adequate control of culicine and anopheline larvae at a dosage of 0.2 lb. DDT/acre and of mosquito adults at a dosage of 0.4 lb./acre was obtained. Subsequently in the Pacific Theater this equipment was adapted to the TBF or TBM Navy torpedo bomber.

In 1945, Husman and Longcoy developed the breaker-bar spray equipment, which had been originally designed and built on Guadalcanal for a Navy TBM airplane. This consists of two spray booms clamped to the wing struts just outside the slipstream of each wing. The boom consists of a $\frac{5}{8}$-inch (inner diameter) tube containing a series of holes from which the solution is emitted. A flat bar is attached $\frac{1}{2}$ inch in front of the orifices to increase the breakup of the spray. This has been adapted to the L–4, L–5, PT–17, and TBF aircraft. A wider swath and more uniform distribution of the spray were obtained with this device than with the original Husman-Longcoy unit; in addition, the average particle size and size range were decreased. Field tests showed that this equipment gave adequate control of both adult and larval mosquitoes.

Exhaust Generators

A simple exhaust-generated smoke-spray device fitted on a Cub airplane was used in a few tests against adult mosquitoes on the Florida Keys. This equipment consisted of a specially designed exhaust pipe into which DDT-oil solutions were injected just below the exhaust flame. This device produced a dense smoke as well as a liquid spray, but it was believed that the

smoke did not contribute appreciably to insect kill because of the small particle size. Division Ten undertook the development of this equipment. In early experiments with a straight exhaust pipe, a higher pressure than was desirable for satisfactory engine performance was developed. To obtain a high-velocity gas flow without back pressure, a venturi was employed as an extension of the exhaust pipe and the insecticide solution was injected into its throat. With this device, an aerosol with a mass median diameter of less than 50 μ may be produced, although the particle size may be increased to a coarse spray by changing the flow rate. In co-operation with the Tennessee Valley Authority, exhaust equipment was installed on a 4–DX Stearman plane with a 450-h.p. engine. Routine anopheline larviciding was carried out by the TVA, and excellent results were obtained at a dosage of 0.1 lb. DDT/acre. An exhaust generator was also designed for the PT–17 Stearman (220-h.p. engine). As a result of the performance shown by the Stearman equipment, this generator was adapted to the Navy TBM. Later, similar equipment was designed for use with other military aircraft (SB2C–4, B–25, and C–47). Entomologic evaluation of the TBM exhaust generator in Florida and Panama showed good control of adult and larval anopheline this equipment, when jungle warfare was no longer in progress, and it was mosquitoes at dosages of 0.3 to 0.4 lb. DDT/acre. The Navy later abandoned believed that aircraft sprays were more suitable than aerosols for application in the open.

Further work is necessary to evaluate aircraft spray equipment. Such tests have been carried out in Florida, where a comparison was made between the straight discharge pipe on a B–25 plane and the exhaust generator on a C–47 plane. The results indicated that at dosages of 0.3 and 0.6 lb. DDT/acre these two equipments give equal control of mosquitoes within twenty-four hours. Somewhat more rapid kill of adult mosquitoes was obtained with the exhaust generator. The fact that an aerosol is less destructive to other forms of life than to mosquitoes should make this method of dispersal especially suitable for peacetime uses. Preliminary design of the exhaust generator was made for an L–5, which is a small maneuverable plane, but no installation has been made on this aircraft.

Dropping of DDT Dispensers from Aircraft

In 1944, some work was done on developing equipment to be dropped from airplanes over jungles; on contact with the ground the insecticide would be discharged. Paper bags containing DDT dust were not a promising means, but liquefied-gas dispersers gave fair results. This work was done in co-operation with Wright Field. A plastic bomb was also in process of development by Division Ten, but with the cessation of hostilities work on this device was discontinued.

PHYSICAL METHODS OF FIELD ASSESSMENT

In the physical evaluation of dispersal methods it was necessary to develop new technics. Several groups of investigators were involved in developing technics for measuring drop spectrum, air-borne dosage, and ground deposition. Several methods that could be used successfully for measuring the particle size of dispersed DDT solutions have been evolved. Waved slides have been employed successfully, provided that consideration is given to the inability of the small drops to impact efficiently on the slides. For more accurate measurements where small particles are being used, the Cascade Impactor has given good results. Settling slides that pick up all sizes of drops with equal efficiency have also proved of value. In addition to clean glass slides, both oleophobic and magnesium oxide coatings were used at different times. The magnesium oxide, which provided a record of the crater formed by the impacting drop, gave good results even with volatile solvents so long as the diameter of the drop was greater than 20 μ. Oleophobic slides, on the other hand, gave accurate results for even smaller drops but could not be used with volatile solvents or, in most cases, when an emulsifier was present. Workers at the University of Chicago Toxicity Laboratory have suggested vertical wires of different diameters for the determination of the mass median diameter of the aerosol cloud.

For measuring air-borne doses, a method was devised in which horizontal slides were employed. This method was dependent on the fact that the number of drops that would deposit from an aerosol cloud was a function of the rate of fall of these drops and consequently, according to Stokes's law, of their diameter. Deposition data have been obtained by counting and measuring the drops on a glass slide, by analysis for DDT, and by the inclusion of dye or radioactive tracers.

CHAPTER XLIV

THE DEVELOPMENT OF NEW INSECT REPELLENTS

ROY O. SCHOLZ

Prior to the wartime program a few investigators were making a search for chemicals capable of repelling insects and suitable for application to the human skin, but it took the impetus of war to stimulate a co-ordinated, systematic search. In 1941 the Army, recognizing the need of improved methods for combating insects attacking man, requested the Department of Agriculture to formulate plans for improving insect control. The resulting program included a study of insect repellents for controlling insects of medical importance.

The objective of the insect-repellent program was to find a nontoxic preparation that once applied to the skin would repel disease-bearing insects for 12 hours. This criterion was chosen because it was necessary to give protection throughout the night, the period when anopheline mosquitoes usually bite.

Early in 1942, with the help of OSRD(CMR) funds, the Orlando, Florida, laboratories of the Department of Agriculture were enlarged for expanded investigations of insect repellency as well as other aspects of insect control. The Food and Drug Administration of the Federal Security Agency, which had been advising the Army on problems connected with the toxicity of insect repellents to human beings, was drawn into the program so that promising repellent chemicals could be quickly examined for their toxicologic effects when applied to the human skin.

The original purpose of these investigations was to screen large numbers of compounds in a search for a nontoxic, long-acting repellent. The screening technics were designed accordingly. At first essential oils such as citronella were tested, and then the few pure chemicals already on the market as insect repellents. Among these, butyl carbitol acetate was found to be a useful repellent and, in addition, had the interesting feature of being nearly odorless. Although this compound was later discarded because of its toxicity to human beings, it did emphasize that chemicals odorless to man could be good repellents against insects. This stimulated the quest, and an extensive empirical examination was made of compounds already synthesized and commercially available. This initial program resulted in the adoption by

the Army of Rutgers 612, Indalone, and dimethyl phthalate as military-issue repellents.

Recognizing the need for an extension of the search for repellents, the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine of the Department of Agriculture instituted a program for the synthesis of likely repellent candidates. The project included investigation of the correlation between chemical structure and repellent activity. As the war progressed, the need for a better repellent became more pressing. In the spring of 1944 the Army requested an expansion of the synthetic and testing programs, and in response contracts were given to several university groups for the synthesis of potential repellents.

The rapid developments that took place not only in the field of insect repellents but also in insect control in general, coupled with the expanding interest that these developments aroused in the various government agencies, led to the need for some co-ordinating group. This resulted in the formation, in 1944, of the Insect Control Committee of the Office of Scientific Research and Development. This committee reviewed the previous work on insect control done in this country and England and sponsored a program for extending and amplifying the lines of investigation already drawn. With respect to insect repellents, the new program emphasized the following aspects:

(1) The development of better repellents and more effective formulations of known repellents in order to improve the control of insect-borne diseases prevalent in the armed forces.

(2) A search for better solvents for insect repellents.

(3) The continuation of studies on the toxicity of repellents and their vehicles.

(4) The institution of basic studies dealing with the natural repellency or attraction of the individual for specific insects.

(5) The extension of investigations concerned with the mechanism of the action of effective repellents.

(6) Frequent meetings at which problems encountered with the use of repellents in the field were to be discussed with representatives of the armed services and civilian investigators.

Integration and co-ordination of a large part of the insect-repellent program took place under the sponsorship of the *ad hoc* Subcommittee for the Development of Repellent Preparations for Skin Application. This group included representatives from the pharmaceutical firms, which prepared formulations and worked in close co-operation with the program.

It became apparent that the repellency test methods used at Orlando, while satisfactory for primary screening to eliminate completely ineffective candidates, were not sensitive enough to detect small differences between repellent preparations. In testing a candidate at Orlando, 1 cc. of the material was applied to a standard area of the forearm of a volunteer, which was then

inserted in a cage containing several thousand mosquitoes. If no bites were received, this procedure was repeated at fifteen- to thirty-minute intervals until the first bite occurred. Great difficulty was experienced in reproducing experiments of this type, owing to a number of factors, both known and unknown. Temperature, humidity, the age of the mosquitoes, the season of the year, individual physiological differences among volunteers — these were but some of the factors influencing the test results. Later, because of the difficulty of comparing the results of tests performed on different days, dimethyl phthalate was adopted as a standard repellent, and paired tests were then made between the standard and the candidate repellents. These tests were much more valuable for a rough comparison of repellents.

Benefiting from the experiences of the Orlando group, the Naval Medical Research Institute developed a sensitive repellency test, which gave remarkably consistent results. In this test the temperature and humidity were controlled and the human subjects were kept under uniform conditions throughout the experiment. Only fresh, rested mosquitoes were used, and thorough precautions were taken to prevent contamination of their cages with residues of the compounds tested. This test procedure was necessarily more complicated than the Orlando method and therefore did not permit the testing of so many compounds.

When the Orlando laboratories had incorporated these improvements into their test procedure, both laboratories were able to secure similar and consistent results. The use of a uniform control preparation with each assay of a new agent made it possible to establish the difference in effect between two or more effective repellent preparations, even when this difference was small.

Although the goal of a twelve-hour repellent suitable for skin application was not reached, many compounds were found that were superior to those previously used. In addition, repellents were discovered that were effective for over a month when applied to cloth; much collateral information was likewise obtained regarding methods of cloth impregnation.

New synthetic candidate repellents to the total of two thousand, two hundred and nine were prepared under contracts with the National Defense Research Committee. The major part of these were entirely new compounds never before synthesized. The Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine furnished the Orlando station with approximately eight hundred and fifty candidates, of which about half were synthesized for the program. The Regional Research Laboratories of the Department of Agriculture furnished approximately four hundred candidates, and the Universities of Illinois and Wisconsin contributed approximately two hundred compounds each. Many industrial concerns also submitted candidates.

With the establishment of the Co-ordination Center of the Insect Control Committee, a catalogue of the repellent chemicals was elaborated. This cata-

logue assisted materially in the efficient functioning of the repellent program, as did the Abstract Bulletin circulated by the Co-ordination Center.

Well over seven thousand repellents and mixtures were tested for repellency at the Orlando laboratories; of these five thousand were tested as skin applications and twenty-five hundred as cloth impregnations. Tests on skin against *Aedes aegypti* revealed approximately four hundred compounds that gave complete protection against this species for over 180 minutes. Very few repellents were found that were effective against *Anopheles quadrimaculatus* for this period of time. Field trials in the Pacific area later indicated that the wild anophelines of these regions are more like *Ae. aegypti* than like *A. quadrimaculatus* in their reaction to repellents.

On the basis of the early repellency and toxicity data, the 1:1:1 and later the 6:2:2 mixture of dimethyl phthalate Rutgers 612 and Indalone were adopted by the Army, and large quantities of these preparations were procured and issued to soldiers in the field. Because of other methods of mosquito control that were in effect simultaneously and because of the variations in enforcing the use of repellents, it was impossible to evaluate the specific effect of these preparations on the disease rate. It was clear, however, that they lessened the pest effects of mosquitoes and flies. In 1945 the results of the new repellent search were reviewed, and the following compounds or mixtures of compounds were selected on the basis of effectiveness, lack of toxicity, and availability as being among the better repellents against *Ae. aegypti*: 2-phenylcyclohexanol; dimethyl ester of cis-bicyclo (2,2,1)-5-heptene-2,3-dicarboxylic acid; 2-phenylcyclohexanol (70%) plus 2-cyclohexylcyclohexanol (30%); cyclopentyl ester of 1-hydroxycyclohexane carboxylic acid; N-sec.-butylphthalimide; and ethyl N,N-di-*n*-propylsuccinamate.

Although a great amount of data had been accumulated on the duration of repellent action by various compounds, it was almost impossible to analyze the data and ascribe a definite repellent time to any given formulation. In the Orlando test the compounds mentioned above all averaged between 50 and 100 minutes before the first bite when tested against *A. quadrimaculatus,* and between 200 and 300 minutes when tested against *Ae. aegypti*. These compounds may be contrasted with oil of citronella, which repels *Ae. aegypti* for 24 to 30 minutes. At the Naval Medical Research Institute the compounds mentioned above repelled *Ae. aegypti* for 133 to 289 minutes when tested under controlled conditions involving high temperature and humidity.

The procedure used by the Orlando laboratories in testing repellents on cloth consisted of impregnating a thin piece of cloth with the preparation and placing it on a person's arm. The arm was then inserted in a cage containing adult mosquitoes, which were forced to penetrate the cloth in order to reach the skin. Such a test was repeated daily until biting had occurred. Of the twenty-five hundred candidates thus tested, many showed complete

repellent action up to 30 days. Among the better compounds tested for preparation by cloth impregnation were piperonal, n-butyl sulfone, and N,n-propylacetanilide. When the military-issue repellents safe for skin application were applied to cloth, complete repellency for about 7 days resulted.

It was found early in the program that the duration of repellency of many chemicals could be lengthened by incorporating them in pastes. A subgroup composed of representatives of various pharmaceutical companies therefore attempted to prepare paste formulations that would be cosmetically acceptable, in accordance with the request of the Army. It was finally concluded, however, that repellency time could not be improved by formulations that were cosmetically acceptable.

In the early part of the program the search for new repellents was pursued along empirical lines only. As the program progressed it became more and more evident that fundamental research was needed on the factors responsible for attraction and repellency.

The Ohio State University group, in reviewing briefly the relation of chemical structure to repellency, pointed out that glycol and hydroxyester repellents are among the most effective. The hypothesis was advanced that this effectiveness may be due to the formation of hydrogen bonds between these compounds and surface moisture, thereby decreasing the vapor pressure of the water. This was correlated with observation that humidity factors are important in attracting mosquitoes.

In a similar discussion the Harvard University group made the observation that mosquito repellency is associated with certain families of compounds. Thus, the most active groups of compounds investigated by them were 1,3 diols, cinnamic and furylacrylic esters, o- and p-alkoxybenzaldehydes and benzyl alcohols, beta-phenylethanols, and N-substituted anilides and amides.

The University of Maryland investigators analyzed the repellency data with respect to the physical properties of the compounds involved and concluded that in selecting a candidate repellent consideration should be given to the boiling point and number of functional groups. In general they showed that a boiling point in the range of 90–130°/0.5 mm. was optimal and that the compound should contain several functional groups. It was postulated that the factor limiting repellent time is the rate of absorption of the repellent by the skin rather than its volatility or chemical structure.

In laboratories at Ohio State University and the University of Pennsylvania some significant advances were made in an understanding of the basic mechanisms by which repellents work. It was demonstrated that a repellent may act either through an olfactory mechanism or through a tactile mechanism; that a mosquito, without coming in contact with the skin, may be repelled by some compounds; and that other materials are distasteful to the mosquito only after it has landed on the treated surface. The temperature and humid-

ity differentials between the skin and the ambient atmosphere are important factors stimulating mosquitoes to bite; the environmental temperature and humidity are likewise important in influencing the biting rate. Other experiments indicated that Rutgers 612, dimethyl phthalate, and Indalone are neurotoxins that cause deterioration of the lipid nerve sheath; thus it was postulated that some repellents may act on the peripheral receptors of the insect. Workers at Cornell University Medical College investigating the rate and mode of disappearance of dimethyl phthalate from the skin showed that loss of repellency was not due to evaporation from the skin surface, but rather to the absorption of repellent chemical into the skin and the breaking of its film on the skin surface. The vehicle in which dimethyl phthalate is applied can strongly influence these factors. In summary the following principal results were obtained:

(1) Liquid repellents for skin application that were 100 per cent effective for over 3 hours were obtained. In addition, several materials were found that were completely repellent for over 30 days when impregnated into cloth.

(2) Certain correlations were found between the physical properties or chemical characteristics of the repellent candidate compounds and their repellency to mosquitoes.

(3) Several thousand new compounds hitherto undescribed in the literature were developed, and the directions for their preparation and some biologic data were made available.

A most important aspect of the program was the establishment of a co-operative organization, any part of which would have been less effective than the whole. The usefulness of such co-operation of government, private industry, and university groups is evident. It was this combining of allied disciplines and their co-ordination toward common objectives that enabled the program to make the advances that it did. Although born of necessity during the war, the usefulness of such co-ordination of diverse interests for peacetime need is clearly evident. It is only now becoming possible to correlate the chemical properties and physical characteristics of compounds with their insect-repellency effectiveness. Thousands of repellent candidates remain to be analyzed in this light.

The search for new repellent compounds should be maintained, and additional repellency tests should be carried out with additional species of insects and further refinements of the various test procedures. Field tests are particularly needed, since the results against one species in the laboratory cannot be safely extrapolated to cover field conditions and other insects. The difference in the reactions of different insects to the same repellent is a basic biologic problem in the designing of insect repellents, and one about which little is yet known.

THE DEVELOPMENT OF NEW RODENTICIDES

RICHARD A. ORMSBEE

W ORLD WAR II found this country short of many things needed for waging modern war. Many of these concerned the health of troops in foreign lands; others had to do with the protection and maintenance of matériel in countries unfamiliar to most citizens of the United States.

It soon became apparent that rodenticides for the control of rats and other rodents were essential. Familiar and classical diseases of war and famine such as plague and typhus were flaring up in Europe and North Africa. Less familiar but important diseases such as tsutsugamushi fever were being encountered in the Orient. In these and other diseases, the rodent serves as the host for the flea, mite, or tick that carries the disease, and as a reservoir of infection instrumental in carrying the disease to new areas. Rats are also responsible for outbreaks of dysentery and other enteric diseases, by directly contaminating food. In addition to bearing disease, the rat is destructive of many instruments of modern war. Forward observers in the front lines were isolated because rats had interrupted communication by chewing the insulation from telephone wires. Silken panels in parachutes were disastrously weakened by rat urine. Delicate electrical equipment was effectively put out of order by the inquisitive rodents.

Thus it was clear that rodent control was essential to the well-being and efficiency of the armed forces. It was simultaneously realized that this country was short of the common rodenticides then in use, since the war had cut off customary imports.

As a consequence of this situation, the Committee on Medical Research began work designed to discover new rodenticides and to review and correlate existing information relating to rodent control. To co-ordinate these projects, the Rodent Control Subcommittee of the Insect Control Committee, Office of Scientific Research and Development, was organized in October 1944, under the chairmanship of Justus C. Ward of the Fish and Wildlife Service of the Department of the Interior, with adequate membership and liaison representing all government agencies concerned with rodent control.

The broad objectives of this subcommittee were to promote the development of new rodenticides more efficacious than those then available and to encourage research on other aspects of the control of rats, mice, and field

rodents. This work was designed primarily as an aid to the armed forces. High priority was given initially to studies on red squill and alpha-naphthyl-thiourea (ANTU), and likewise to the screening program designed to reveal new rodenticides. In response to the Army's need, immediate steps were taken to speed up the current research and to initiate new research. The search for new rodenticides that was being carried out by the Fish and Wild-life Service was enlarged and speeded up by means of an OSRD(CMR) contract. The National Defense Research Committee, the Chemical Warfare Service, the Fish and Wildlife Service, the United States Public Health Service, and various Army and Navy groups were drawn into the picture, with the result that a co-ordinated program was shortly under way.

One of the first requisites was a survey of the available literature in the field of rat control, which was widely scattered among many journals of different countries. Such a survey was immediately started. The resulting annotated bibliography, of lasting value to the field of rodent control, has been completed and made available to all workers in the field.

Red squill is a rat poison that is manufactured by grinding to a fine pow-der the dried bulbs of *Scilla maritima*. This plant grows in southern Italy and around the shores of the Mediterranean. It was introduced into this country in the 1920's and was immediately widely used, owing to the fact it was not only a potent poison for rodents but was likewise relatively safe, since its unpalatability and emetic properties lessened the hazard of acciden-tal poisoning of man and of other animals. Rats, fortunately, are unable to vomit under any circumstances. When the supply of red squill was cut off by the war, efforts were immediately made to develop methods of growing red squill in this country, and genetic studies on the plant were begun so that a squill bulb of higher potency might be produced. This work was being done by the Bureau of Plant Industry of the Department of Agriculture, with the co-operation of the Fish and Wildlife Service and the Food and Drug Administration. It was hoped that squill could thus be supplied from plantations in this country, and that the yield might be increased as success-fully as was done in the case of morphine production from poppies.

This activity was, of course, well under way when the Rodent Control Subcommittee began its activities. The Subcommittee immediately encour-aged work on the isolation of the toxic constituent, scilliroside, derived from crude red squill, with the hope of devising an accurate chemical-assay method, and with the hope that the information thus gained might enable the synthesis of scilliroside to be economically performed. A chemical assay for scilliroside would alleviate the laborious and unsatisfactory bioassay then necessary. This program, however, rapidly lost its practical significance as the result of the discovery and development of two new synthetic rodenti-cides.

Alpha-naphthylthiourea was developed as a rodenticide in the laboratories

of Curt P. Richter, at the Johns Hopkins University School of Medicine. Richter's original interest had not been that of searching for new rodenticides; his work had been concerned mainly with the perception of taste in rats as applied to a great many compounds of diverse structures. In his "rat cafeteria," an animal could feed on any one of scores of different pure compounds in water solution.

In the course of his investigations Richter noted that some of his experimental animals died as a consequence of eating small amounts of the supposedly nontoxic compound phenylthiourea. This compound has been widely used in the field of genetics and sensory perception, since it was found that the inability of human beings to taste it was inherited as a Mendelian recessive character. The surprising toxicity of phenylthiourea to rats suggested that it might be of value as a rodenticide. Tests with wild Norway rats, however, gave poor results, probably owing to its bitter taste. On the theory that a related compound of similar toxicity but greater palatability might prove more successful, investigations were initiated in which a long series of substituted thioureas were bioassayed against Norway rats. The result was ANTU, which is highly toxic but tasteless to the rat, because it is insoluble in water or in the animal's saliva, and is therefore readily acceptable when incorporated in bait.

Realizing the potential value of this discovery, the Office of Scientific Research and Development thereupon made large amounts of this material available for field testing. The City of Baltimore, under the direction of the City Health Office and Dr. Richter, made extensive tests in the city over a period of four years, with the result that ANTU was shown to be a valuable weapon in the control of the common Norway rat.

The Rodent Control Subcommittee co-operated in the further field evaluation of this compound by distributing it to many agencies, including the Fish and Wildlife Service, the United States Public Health Service, the Army and Navy, and various members of the British Commonwealth. A surprising thing was revealed: ANTU, which is highly toxic to the Norway rat, was virtually nontoxic to every other species of rodent against which it was tested. The black rat, a close relative of the Norway rat, was almost unaffected by doses fifteen to twenty times as great as those guaranteed to kill a Norway rat. This feature of ANTU immediately indicated that while it was of considerable value in the control of Norway rats, it was not the ideal rodenticide for the armed forces, since they required a rodenticide that would be equally effective against all rodents encountered.

At this juncture a new compound of potential value appeared as a result of the screening program being carried out by the Fish and Wildlife Service. One of the many close relationships maintained in this program was that existing between Division Nine of the National Defense Research Committee and the Patuxent Laboratory of the Fish and Wildlife Service. The for-

mer was primarily interested in toxic agents for chemical warfare, a program that had led it into rather extensive investigations of a series of organic fluorine compounds, many of which were highly toxic. Some of these were examined by the screening group at Patuxent, and one of them seemed particularly qualified. It was a quite volatile liquid, however, and thus was not suitable as a rodenticide. Further work produced the sodium salt of this compound, which is a water-soluble, white, colorless, odorless, and highly toxic chemical and thus possesses many of the characteristics of an ideal rodenticide. This substance is sodium fluoroacetate, or 1080.

Preliminary tests disclosed that this compound was highly toxic to laboratory rats and that it was extremely acceptable, being undetected until lethal amounts had been consumed. After these favorable preliminary tests had been reported, the National Defense Research Committee contracted with the Monsanto Chemical Company for an adequate amount of 1080 for further experimental work. The Rodent Control Subcommittee then distributed 1080 widely to the Army, the Navy, the United States Typhus Commission, the United States Public Health Service, the Fish and Wildlife Service, the Texas State Department of Public Health, and various members of the British Commonwealth, through the agency of the British Commonwealth Scientific Office.

Field results from all over the world became rapidly available. The Rodent Control Subcommittee was the instrument for co-ordinating the field and laboratory investigations, collecting and distributing reports, and arranging meetings among the interested parties. These field tests were made possible in part by funds from the Office of Scientific Research and Development and in part by the enthusiastic and substantial co-operation of the several independent organizations concerned. The final result was that 1080 was ready for use by the Army and by other competent organizations, both public and private, within sixteen months after it had first been tested in the laboratory. The volume of laboratory and field data accumulated would have taken three to five years to acquire under normal circumstances.

Although the development of ANTU and 1080 were the two major practical accomplishments of this program on rodent control, many other things of value have accrued. Not the least of these is the information obtained from fundamental studies on the pharmacology, physiology, and biochemistry of 1080, ANTU, and other toxic compounds. This work throws light on possible future paths of investigation and reveals ANTU and 1080 as chemical tools of significance in the hands of physiologists and biochemists. ANTU, for example, has been shown to be a most specific compound in its physiological action. It is poisonous only to Norway rats, and it affects only specific tissues in the body of that animal. The rat, furthermore, may develop great tolerance to ANTU when the compound is fed in sublethal doses. This tolerance can be quickly built up to fifty times the lethal dose

and can disappear almost as quickly. ANTU causes considerable derangement of well-known metabolic processes such as the growth and pigmentation of hair and is intimately connected with cystine metabolism in the body. Likewise, 1080 exhibits a differential toxicity to various groups of animals. Dogs and other members of the canine group, for example, are roughly ten times more sensitive to it than are rodents. In mammals, 1080 produces brain waves indistinguishable from those of petit mal, and this is of possible importance as a research tool in investigations on this disease. The active principle of red squill, scilliroside, is likewise of considerable biologic interest, in that it appears in some way to be connected with steroid metabolism in rats. Female rats are roughly four times as sensitive to red squill as are male rats; yet when' male rats are castrated their susceptibility becomes equivalent to that of the females.

One method of rodent control widely used in some areas is that of fumigation. This may consist of treatment either of whole buildings or of the burrow systems of rodents. The fumigation of burrow systems is of considerable importance in the western United States, where sylvatic plague is carried by ground squirrels, which likewise cause extensive damage to crops. Other methods are often not adaptable for the control of these animals. The Rodent Control Subcommittee was instrumental in establishing a co-operative association of the Chemical Warfare Service and the Fish and Wildlife Service on this problem, with the result that considerable research of mutual benefit is continuing along these lines in both organizations.

During the world-wide field testing of 1080 and ANTU, acquaintance was made with scientific representatives of many foreign countries. These contacts are proving to be of value in postwar co-operative research problems dealing with rodent control. The Rodent Control Subcommittee embarked on a postwar program of encouraging research dealing with rodent control, and close contact is being maintained among the laboratories engaged in this work. In co-operation with the Army Committee for Insect and Rodent Control, samples of new rodenticides discovered in Germany by teams of the Combined Intelligence Operation Services have been synthesized, and field tests on these compounds have been begun.

The information that has been uncovered during the war on the extreme specificity, combined with extreme toxicity, possessed by some compounds makes it not impossible that continued work along these lines will result in a rodenticide that is highly toxic to rodents, comparatively nontoxic to all other forms of life, and therefore an efficient rodenticide that can be used with complete safety by the general public. Such a rodenticide will take the place of red squill, which is now the only rodenticide that can be used in this way, but which by reason of its poor acceptance and relatively low toxicity to rodents cannot be considered ideal. Investigations of the mode of action of toxic compounds, which must inevitably accompany such a search

for new rodenticides, are very likely to shed new light on physiological and biochemical problems quite unrelated to rodent control.

A wide field of contacts with workers in other countries on the common problem of controlling rodents has been and is continuing to be developed. While the major practical contributions of this work have been the development of 1080 and ANTU, another highly significant contribution should be noted. This is the demonstration that co-operative effort in scientific research by many diverse agencies and individuals is capable of immensely accelerating the pace of scientific advance that is necessary in the modern world.

Part Seven: Adrenocortical Steroids

CHAPTER XLVI

THE SYNTHESIS OF ADRENOCORTICAL STEROIDS

T. F. GALLAGHER

THE PROGRAM of research directed toward the synthesis of adrenocortical steroids was initiated by the Committee on Medical Research as the result of physiological research indicating that these hormones or their chemical relatives would be of value in aviation medicine and in the treatment of shock, fatigue, and related conditions. A critical evaluation of the therapeutic properties of the hormones was possible only if abundant supplies of the pure chemical compounds could be made available for clinical testing. To obtain these compounds from adrenal glands in wartime was impossible because the supply of glandular material was limited and the hormonal content of the organ is low. The partial synthesis of the hormones from naturally occurring steroids was therefore the only available course if physiological investigations were to be furthered.

The researches of one investigator were supported by the National Research Council of Canada. Dr. E. C. Kendall of the Mayo Foundation and certain commercial organizations participated voluntarily in the program without contract or support from the Committee on Medical Research. All the other investigators, representing a number of universities and commercial organizations, were under contract.

At the beginning of this project the chemical structure of many steroids obtained from the adrenal cortex was known in its essential details. Moreover, the partial synthesis of one of these, desoxycorticosterone, had been effected on a commercial scale. Three principal problems were unsolved. The first and most important of these was the preparation of an 11-oxygenated steroid that could serve as a suitable material for the synthesis of Kendall's Compound E, corticosterone, and dehydrocorticosterone. Allied

with this problem was the related one of removing the steroid side chain by methods suitable for large-scale production. When these had been solved there remained the problem of synthesizing the carbohydrate-like side chain of the cortical steroids in an 11-oxygenated compound. On the biologic side it was desirable to have standardized methods for the evaluation of pure compounds as well as of impure glandular extracts.

In this brief report no attempt will be made to credit the individual investigators and their collaborators with specific results, since many of the experiments have been published and others will doubtless soon appear in the literature. Instead, an attempt will be made to record the development of the whole problem from its initiation.

STUDIES ON PLANT STEROIDS

Since ergosterol was an especially promising substance for side-chain degradation and at the same time, because of its nuclear unsaturation, might prove suitable for the introduction of oxygen at C_{11}, the removal of the side chain was investigated. It was possible to eliminate it in relatively few steps and in high yield, but the procedure was complicated by the difficulty encountered in removing the maleic anhydride used to protect the conjugated unsaturation. The introduction of oxygen at C_{11} in the form of a ketone group was possible, but unfortunately under the experimental conditions an aromatic ring B was formed. Numerous other experiments with ergosterol and its derivatives yielded interesting information, but have not as yet offered a practicable solution to the problem.

Other investigations on spinasterol, zymosterol, and cafesterol provided information of value to steroid chemistry, but furnished no practicable route to the synthesis of cortical hormones.

STUDIES ON DESOXYCHOLIC ACID

REMOVAL OF THE SIDE CHAIN

Initially considerable effort was devoted to studies on the feasibility of removing the bile acid side chain in one step by direct oxidation. This indeed proved possible, and both etiodesoxycholic and 3,12-dihydroxyetiocholan-17-one were obtained. Under the various conditions investigated, however, extensive rupture of the nuclear ring system occurred, and the yields were therefore poor. Transformation of bile acids to substances more nearly resembling cholesterol and one-stage oxidation of these products likewise proved disappointing.

Striking improvements were effected in the side-chain removal by the classical Barbier-Wieland procedure. These studies, on a variety of different

compounds, have made possible the preparation of the hormone in acceptable yield. Several alternative procedures were investigated, with promising results. Among these were the degradation of the silver salts of bile acids by treatment with bromine to form the alkyl bromide and dehydrobromination with an organic base, followed by ozonolysis of the alkene. A similar degradation used the methyl ketone derived from the carboxyl group, followed by bromination, removal of hydrobromic acid, and oxidation. A novel method, since discovered independently by Miescher and his collaborators in Switzerland, was developed in which the diphenylethylene, obtained from the bile acid ester with the Grignard reagent, was brominated with bromsuccinimide alpha to the ethylenic bond by the procedure of Zeigler and his co-workers. Removal of the elements of hydrobromic acid yielded a diphenylbutadiene derivative which on oxidation yielded the pregnane ketone.

Several investigations of methods for transforming the pregnane ketone to either the etio acid or the 17-ketosteroid were made. These included a study of the haloform reaction in several variations, oxidation of the benzilideneacetone derivative of Hoehn and Mason, and direct oxidation of 1,1-diphenyl-methyl-(3,12-diacetoxy etiocholanyl)-ethylene. These studies resulted in significant improvement of the yield; since all the details have not been published an exact evaluation is not possible.

A procedure for the conversion of the bisnor acid to the 17-ketone by means of the Curtius rearrangement was investigated in detail. The mixture of pregnenes and alcohols resulting from diazotization of the 20-aminopregnane was converted by ozonolysis and permanganate oxidation to both the etio acid and the 17-keto derivative. These reactions were studied on 3-hydroxy-11-keto bisnorcholanic acid, and the products obtained served as intermediates in the synthesis of Kendall's Compound E and of dehydrocorticosterone.

Preparation of Steroids Oxygenated at C11

Prior to the initiation of the project, a method for the preparation of Δ^{11} lithocholenic acid had been independently developed, making use of methods essentially similar to those since published by Reichstein and his colleagues. The Swiss reports were, however, greatly delayed and were unknown to the conference. This product was of great value in the study of the chemistry of ring C. Its use led directly to the development of two procedures for the formation of an 11-oxygenated steroid and indirectly to another procedure. Similarly $\Delta^{9,11}$ lithocholenic acid, which had been prepared before the war, served in the study of ring C chemistry and was utilized in the development of a method for the preparation of an 11-keto steroid.

The method used in transforming both Δ^9 and Δ^{11} lithocholenic acid to the 11-keto acid consisted in the addition of hypobromous acid to the

ethylenic bond, followed by oxidation of the hydroxyl group and reductive removal of the halogen. An alternative procedure utilized the epoxide prepared from the Δ^{11} acid, which on acetolysis, followed by oxidation and Wolff-Kishner reduction, yielded the 11-hydroxy derivative.

Another procedure employing the $\Delta^{9,11-12}$-keto derivative of desoxycholic acid, for which a greatly improved method using selenium dioxide oxidation was developed, was investigated extensively and proved extremely successful. This substance was partially reduced to the 12-hydroxy derivative, which on treatment with hydrogen bromide was converted to the 12-bromo derivative. The latter underwent an unusual allylic rearrangement, with the formation of a 3,9 epoxide and, most important for further work, an ethylenic linkage between carbons 11 and 12. It was possible to convert this to an 11-hydroxy-12-bromo derivative from which the halogen could readily be removed. The interesting product was especially suited for side-chain degradation, since the 3-hydroxyl had been transformed to an inert epoxide and since the 11-keto group, owing to steric hindrance, was not attacked by the Grignard reagent. The epoxide could be restored to the original 3-hydroxyl group by treatment with hydrogen bromide; in this reaction the length of the side chain markedly altered the reaction.

Two similar methods for the introduction of an 11-oxygen function differing from those already described were discovered. The 12-keto derivative of desoxycholic acid was brominated in the 11 position, and hydrolysis of the halogen with aqueous base at low temperature yielded the 11-hydroxy-12-keto compound. In one procedure this was directly transformed by Wolff-Kishner reduction to 3,11-dihydroxycholanic acid. In the other procedure oxidation yielded the 11,12-diketo derivative, which formed a monosemicarbazone. Wolff-Kishner reduction of this derivative yielded 3-hydroxy-11-ketocholanic acid. In the course of these investigations several puzzling problems in the chemistry of ring C were elucidated.

Synthesis of the Cortical Hormone Side Chain

For the preparation of dehydrocorticosterone and the C11 epimer of corticosterone the general methods developed by Reichstein and his co-workers were found adequate. Although the synthesis of corticosterone was not accomplished, considerable research was done on the use of lead tetraacetate as a means of achieving the ketol side chain of this substance. Similar studies have since been published by the Swiss workers.

The synthesis of the dihydroxyacetone side chain of Kendall's Compound E was accomplished from 3-hydroxy-11,17-diketoetiocholane. Since this work has been published in detail, it can be summarized as follows. Acetylene added only to the 17-ketone yielded a 2-carbon side chain. Through several intermediates this product was transformed to Δ^{17} pregnene-3,21-

diol-11-one, which was oxidized to the 3-keto derivative with suitable protection of the primary alcohol. This product when reacted with osmium tetroxide yielded a trioldione, which was converted to the α-β unsaturated ketone in ring A. This yielded a monoacetate at C21 and under carefully controlled condition was oxidized to the acetate of Compound E.

BIOLOGIC INVESTIGATIONS

Two methods that are satisfactory for the assay of adrenocortical extracts were developed. Various crystalline steroids and glandular extracts were assayed and evaluated in terms of two qualitatively different effects. Standards were suggested for both methods.

SUMMARY

The primary objectives of the conference were achieved by the development of methods for transforming a bile acid to an adrenocortical hormone. These involved studies on the removal of the side chain of the bile acids and sterols by original and by classical methods in order to facilitate large-scale production. The original research necessary to devise methods for introducing oxygen at position 11 of the steroid nucleus resulted in the development of no less than six procedures for this purpose. Finally, methods for the synthesis of the cortical hormone side chain were investigated and applied to C11-oxygenated steroids.

Part Eight: Malaria

CHAPTER XLVII

INTRODUCTION

GEORGE A. CARDEN, JR.

WHEN the supply of quinine was suddenly cut off by the Japanese attack on Pearl Harbor in December 1941, the Army, Navy, and Marine Corps faced a deadly serious problem. A long war in the most malaria-infested areas in the world lay before them, and they were deprived of their only reliable therapeutic weapon, quinine. In the late spring of 1940, a year and a half before Pearl Harbor, the vital importance of malaria as a military problem and the necessity for research looking toward better means for its prevention and cure had been stressed by the Surgeons General of the Army and Navy.

At its first meeting on July 31, 1941, the newly created Committee on Medical Research of the Office of Scientific Research and Development made the decisions that were to link its own plans with those of the National Research Council. The Office of Scientific Research and Development established a broad, flexible contract with the National Academy of Sciences whereby the Academy, represented by the National Research Council, was granted adequate funds and facilities for maintenance of advisory committees and administrative offices. With this start, the organization of malaria workers and consultants began to develop.

Progress in the field of chemotherapy had received great impetus through the advent of the sulfonamides, and technics to improve the effectiveness of these agents were being developed. It had been shown experimentally that the action of a sulfonamide against an infection in the body is dependent on an adequate and sustained concentration of the drug in the blood, and clinical investigators throughout the country had demonstrated beyond doubt the practical value of this discovery in the treatment of bacterial infections. The knowledge thus gained in the sulfonamide field was applied in the launching of a chemical attack on malarial parasites, and the men chosen by the Committee on Medical Research to conduct this study

were among those most familiar with these new developments in chemotherapy.

By June 1942, there had been formed the nucleus of an integrated program. Although only one group of chemists was under contract to synthesize potential antimalarial compounds, there were a number of OSRD (CMR) contracts with institutions for their pharmacologists and parasitologists to test compounds against malarial infection in ducks, chicks, canaries, and monkeys. Also, one group of investigators was exploring cautiously in man any compound that showed sufficient promise as an agent against the bird and monkey infections. At the same time, a program of basic research in the fundamental mechanisms of the disease was initiated. This included histologic studies on the life cycle of the parasite, biochemical studies on enzyme systems in the metabolism of the parasite and on its nutritional requirements, and serologic studies on immunity.

To facilitate the cataloguing and distribution to testing laboratories of the swelling tide of compounds sent in as voluntary contributions from commercial and university laboratories, in addition to those synthesized by the Committee on Medical Research chemists, the Office of the Survey of Antimalarial Drugs was formed, through a contract between Johns Hopkins University and the Office of Scientific Research and Development. This office has performed one of the most important functions in this entire program in issuing periodically tabulated data on the results of tests in the various laboratories. The analysis of such data was the means by which this program took direction. Therefore, the Survey Office became the central depot for the receipt and dissemination of technical information on each compound tested, including source, chemical structure, physical properties, results of screening tests, and so forth.

While the search for better antimalarial drugs was being energetically expanded, the Army and Navy were committed to the use of quinacrine. Disturbing reports were received suggesting that American-made quinacrine was impure and ineffective, and that it contained a toxic fraction not present in the original product synthesized by the Germans. Similar reports were received by the British in relation to mepacrine (British quinacrine). An extensive exploration of this problem, which continued over a period of five months, settled the question on irrefutable scientific grounds. It thus enabled the Army and Navy and the British authorities to proceed with the procurement of quinacrine with the absolute assurance that American and British quinacrine were fully as pure as the German product, and that any toxic reactions that might be encountered would be the result of the action of the quinacrine molecule itself and not of any contaminating or adulterating substances.

While this alarming rumor was being buried, reports were received from the Army indicating that the use of suppressive doses of quinacrine was

accompanied by vomiting and diarrhea in a high proportion of cases, and that troops were refusing to take the drug. Furthermore, many cases of malaria developed in men who were on the recommended regimen of suppressive quinacrine therapy. Even in the treatment of the acute attack, the drug was often reported to be slow and inadequate in terminating the attack. At the urgent request of the Surgeons General of the Army and Navy, our investigators undertook to study these problems and to find the most satisfactory means of employing quinacrine in suppression and treatment.

Precise information was obtained on the absorption and excretion of quinacrine in animals and man, the extent to which this drug is destroyed by the metabolic processes of the body, its distribution between red cells, white cells, and blood plasma, the extent to which it is bound to tissues and to plasma proteins, and the relationship between oral dose, plasma level, and therapeutic effect. A highly accurate, yet practical method for measuring the concentration of quinacrine in plasma at levels as low as 5 μg. per liter was devised. The acute and the chronic toxicity of the drug in animals and man were accurately defined.

It was clearly shown that both benign tertian malaria (caused by *Plasmodium vivax*) and the malignant tertian variety (from *P. falciparum*) could be effectively suppressed by quinacrine if certain minimal plasma drug concentrations were continuously present. Therefore, schedules of drug administration for use in the field were drawn up that guaranteed the maintenance of such protective concentrations with no loss of effectiveness from toxic symptoms. Furthermore, the significant discovery was made that quinacrine accumulates slowly in the blood plasma, so that in field operations suppressive treatment must be begun two or three weeks in advance of exposure to malaria, or larger priming doses must be given to secure adequate protection. These basic observations on the behavior of quinacrine in the body immediately became the foundation of today's highly effective use of the drug as a malarial suppressive in the field.

Great strides were also made in the treatment of the acute attack of malaria. By applying the knowledge gained from the laboratory studies, it became possible to terminate malarial fever within twenty-four to forty-eight hours and to destroy the parasites in the circulating blood. It then became apparent for the first time that quinacrine, when properly administered, was vastly superior to quinine. Furthermore, when these new principles of quinacrine therapy were applied to the treatment of *falciparum* malaria, the only type of malaria that carries a high mortality, it was found that the disease could be prevented or cured with quinacrine, whereas with quinine it could only be suppressed. (The incidence of *falciparum* malaria varied in different localities. In the Southwest Pacific it was approximately 50 per cent, in West Africa 90 to 100 per cent, and in the China-India Theater 60 per cent.) In consequence, the death rate in the American and

British armies dropped to less than one out of every 2500 cases, whereas that in the Japanese Army continued high.

With quinacrine established as an effective suppressive and therapeutic agent, the urgent need for a quinine substitute was superseded by equally pressing problems. The Army and Navy were quick to point out that the tactical advantage gained from the suppressive use of quinacrine was a two-edged sword. Since this drug effectively suppresses but does not cure *vivax* malaria, it was apparent that the Army and Navy and the Veterans Administration might eventually be faced with the problem of caring for many hundreds of thousands of men seeded with the disease, who would develop full-blown attacks as soon as they returned to this country and stopped taking their daily doses of quinacrine. Moreover, these attacks would probably continue from one to three years, for it was known that suppression even for as long as two and a half years does not influence the ultimate course of the disease.

The Committee on Medical Research promptly accepted this challenge and encouraged expansion in the field of malaria research in the hope of discovering a truly curative agent. In order to co-ordinate the work in this field, the Subcommittee on the Co-ordination of Malarial Studies was established under the Committee on Medicine of the Division of Medical Sciences, National Research Council, and held its first meeting on January 20, 1943. Three panels were established under the Subcommittee — the Panel on Synthesis, the Panel on Pharmacology, and the Panel on Clinical Testing. In May of the same year, a fourth panel was added — the Panel on Biochemistry.

In the late fall of 1943, in order to establish closer contact with the malaria problem in the Army and Navy and to facilitate exchange of information, there was formed a joint Board for Co-ordination of Malarial Studies, with equal representation from the Office of Scientific Research and Development, the Army, the Navy, the United States Public Health Service, and the National Research Council. To the four panels named above was added the Panel of Review, which served as executive committee of the Board. The formation of this board marked the beginning of an integrated, well-rounded program, which has developed with rapidity and effectiveness.

To aid in the administration of the growing number of malaria contracts then in force, the Committee on Medical Research established a Division of Malaria in April 1945. The Executive Secretary of the Board for Co-ordination of Malarial Studies was made chief of this division, an appointment that served to integrate the work of the Board closely with the Committee on Medical Research.

The procedure for testing antimalarial compounds was developed in accordance with the following plan. Pursuing rational leads as they de-

veloped, organic chemists prepared new compounds in small quantities. These compounds were then screened for activity in various forms of bird malaria. Those that showed particular promise were prepared in larger quantities and were put through the various procedures necessary to define their use in man. First, the toxicity in animals was studied extensively; next, methods for quantitative estimation in body fluids and tissues were devised, and the absorption, distribution, and excretion of the compound were studied quantitatively in animals. If animal toxicity was not prohibitive, the drug was studied in the clinical testing units. At first it was administered cautiously to volunteers (conscientious objectors or penitentiary inmates) to determine its absorption, excretion, and distribution. During these preliminary explorations, the chemical method for measuring concentration in body fluids was perfected for practical application in the clinical trials to come. When all the above information was in hand, the critical test was made to determine with precision the antimalarial activity of the new compound in human subjects, by comparing it quantitatively with a standard antimalarial drug; that is, quinine, quinacrine, or pamaquine.

The test in man was conducted against one of three strains of *vivax* malaria or against one of two strains of *falciparum* malaria, or both. The initial tests were designed to determine the effect of the new agent against the parasites in the circulating blood and against the several stages of the parasite in the tissues. In the former test, the subject was given malaria by the injection of blood infected with malarial parasites; in the latter, the infection was induced by the bite of an infected mosquito. The drug could then be tested prophylactically or curatively. If the prophylactic test was negative, the drug could be tested for its curative action in the same subject by initiating treatment after the disease was established. In this way, the selective activity and many other important effects of an entirely new chemical compound were accurately determined in man.

When a compound had shown particular promise as a suppressive, prophylactic, or curative agent, it was subjected to extensive toxicity and tolerability studies in volunteers prior to recommending it to the Army or Navy for a limited trial on military patients with malaria. Once a compound had finally reached the stage of trial by the Army or Navy, adequate quantities of the material were prepared under OSRD(CMR) contract or through commercial concerns. The subsequent studies in service installations were guided and directed by the Board for Co-ordination of Malarial Studies, and additional quantities of material were supplied whenever further expansion of these studies was indicated.

One of the important functions of the Board was to effectuate a wide exchange of information between its members and members of its panels, the OSRD(CMR) investigators, the interested parties in the Army and Navy, and allied scientists in England, Australia, Canada, India, and Russia. This

was accomplished by wide and prompt distribution of all reports of progress at the different levels of investigation.

In order to envisage the scope of this program without detailing the accomplishments achieved therein, it may be well to note that over fourteen thousand compounds were studied chemically and pharmacologically. They were tested in one or more species of avian infections, and approximately one thousand of these compounds were tested in one or more species of mammals for toxicity and general pharmacology. Intense efforts were made to co-ordinate the relation between the action of a compound and its chemical configuration. Although the complete answer was not found, interesting correlations of the accumulated data, which may bear fruit in the future, were brought to light. Likewise, intense efforts were made to understand the basic biochemical and biologic characteristics of the various malarial parasites. Although not all the questions were answered, much knowledge was gained that not only throws light on the basic biology of this disease but may also add to knowledge in other lines of investigation.

Over one hundred new, untried chemical substances were studied in man for antimalarial activity, physiological distribution, and toxicity. Of these, a score or more proved to have favorable prospects as useful agents in either the suppressive treatment or the cure of malaria. Many of the most promising of these compounds were developed too late in the program, however, to permit extensive exploration. In future studies on this disease, many of the unfollowed leads and current loose ends will undoubtedly be explored, and additional compounds of value will most probably be developed.

Although malaria as a disease has not been erased from the face of the earth, the knowledge gained as a result of this concentrated attack by a small army of scientists has greatly reduced the extent of the malaria problem. The disease can be effectively cured or suppressed for indefinite periods of time. This is the concrete accomplishment of these investigations. The other and possibly more valuable achievements, although less tangible, may be more profound. These include new light on the behavior of chemical substances in the human body, on the relation between chemical action against the infecting organism and the chemical configuration of the molecule of the drug, some slight insight into possible mechanisms of action of chemical compounds on living organisms, and a more complete understanding of the chemical and biologic aspects of toxicity.

In the following chapters the different levels of investigation will be described, and from these accounts some of the thought behind the accomplishments may be gleaned.

THE SYNTHESIS OF ANTIMALARIAL DRUGS

ROBERT C. ELDERFIELD

THE PROGRAM of the Committee on Medical Research in the synthesis of new antimalarial drugs probably represents the most concentrated massing of chemical talent ever brought to bear on a chemotherapeutic problem. During the last year of the war, thirty groups in twenty-seven university laboratories and one industrial laboratory were engaged in preparing new substances to be tested against the various malarias. Approximately two hundred and fifty chemists were employed on these contracts. In addition, three other industrial contracts were set up to take care of such development problems as arose in connection with the translation of laboratory processes to large-scale operation. Further, through the wholehearted co-operation of the pharmaceutical industry, many of the leading drug manufacturers carried on extensive programs on the synthesis of new drugs under their own auspices, submitting their products to our laboratories for testing.

How many chemists were thus engaged it is impossible to say. The efforts of both groups were pooled to form an effective operating entity, with the common objective of discovering more effective antimalarial drugs than those heretofore known. Hopes of acquiring personal fame or benefit were put aside, and the thought uppermost was how the individual could contribute most effectively to the success of the undertaking.

Of the approximately fourteen thousand chemical substances that have been examined for antimalarial properties during the years 1941–45, roughly one third represent new chemical compounds synthesized for the first time. The remainder were chosen from stocks of chemicals that had accumulated on the shelves of laboratories, both academic and industrial, over the years.

The efforts of the chemists engaged in this work were directed and channeled largely on the basis of information forthcoming from the results of the various tests done on substances, either new or old, of known chemical structure. As described in the introduction to this section, such data were collected and correlated in the Office of the Survey of Antimalarial Drugs. The general direction and correlation of the clinical, pharmacologic, biochemical, and synthetic chemical investigation were accomplished through a Panel of Review. The synthetic program was formulated largely in accordance with directives from that panel. Within the Panel on Synthesis, a com-

pact steering committee of four was set up, and through it the detailed organization and direction of the synthetic program were channeled.

Organic chemical compounds are classified on the basis of certain characteristic arrangements of the constituent atoms within their molecules. Thus, a substance consisting of nine carbon atoms, seven hydrogen atoms, and one nitrogen atom arranged in the following fashion is known as

quinoline. In the quinoline molecule, one or more of the hydrogen atoms may be replaced by other atoms, such as chlorine, or by whole groups of atoms known as radicals; for example, the grouping $NHCH_2CH_2CH_2NH_2$. Thus, there arise subgroups in the broad class of quinoline derivatives. Considered from this point of view, some antimalarial action has been found in about seventy classes of organic compounds. This does not mean that all seventy classes represent variations based on quinoline; on the contrary, the basic quinoline structure occurs in possibly five of these classes and the remainder are found among other basic groups analogous to quinoline. The problem then arose of how best to distribute the efforts of the chemists among these seventy classes of compounds.

Drugs were originally selected for synthesis or testing on the basis of one or more of the following criteria.

(1) The substance had been found to be effective against diseases other than malaria, and it was therefore tested against malaria. Among drugs of this class may be cited the sulfonamide drugs and the arsenicals.

(2) The remedy enjoyed a local or old-wives reputation as an antimalarial. Such remedies are usually extracts of plants or concoctions of various sorts and, strictly speaking, do not fall into the province of the synthetic chemist. Examples of this class are extracts of the Chinese plant *ch'ang shan* and a mixture of ground eggshells and whiskey.

(3) The substance was expected to interfere selectively with the basic metabolism of the malaria parasite without interfering with that of the host. Many variants of the essential vitamins had been prepared on this basis.

(4) Finally, studies on the physiological disposition of antimalarial drugs of proved value had pointed the way to the selection of new compounds for testing.

Within each chemical class of compound — for instance, the quinoline derivatives, to use the example already chosen — an almost infinite number of variations is theoretically possible. Thus, each of the hydrogen atoms may be replaced by a single element or radical in turn, or more than one hydrogen atom may be replaced. When substitution of one or more of the hy-

drogen atoms by an entire radical is carried out, an obviously wide variation within the radical is possible when it is remembered that in the radical, as in the basic quinoline molecule, any or all of the hydrogen atoms may in theory, but not always conveniently in practice, be replaced in turn by other atoms or radicals. Each such replacement exerts an effect on the physiological properties of the substance.

When significant antimalarial activity was discovered in any one class of compounds, the efforts of the chemists were concentrated on building molecules related to the basic active structure, with the purpose in mind of improving on properties such as antimalarial activity, toxicity characteristics, physiological disposition, and so forth, which when taken together determine the usefulness of a drug. Throughout the war several such broad classes of substances were simultaneously under intensive synthetic chemical exploration. Exploration of a given group was discontinued when sufficient evidence accumulated to indicate that the achievement of the desired combination of properties was improbable in the time available. In the cases of chemical classes where the efforts of the chemist led to realization of the goal, work was continued in the hope of still further improvement.

The synthesis of an organic compound, even a simple one, involves the combination of various smaller parts of the final molecule. These component parts are known as intermediates. In the exploration of the effect of variants of chemical structure, a demand for relatively large amounts of certain intermediates invariably arises. For example, a given drug can be represented by two radicals A and B joined together. If it is desired to study the effect of variations in radical B, while holding radical A constant, a demand is created for large amounts of radical A, and if several laboratories are engaged on this phase of the work, it is of obvious advantage to have available a common source of A. Many such cases arose during the program, and because of lack of commercial availability of many of the intermediates, the problem of their preparation became critical. To meet this situation, certain of the investigative groups devoted their entire effort, or a major portion of it, to the task of supplying intermediates. Theirs, in some respects, was a thankless chore, since only rarely did they experience the reward of the chemist in receiving a favorable pharmacologic report on a drug submitted for testing. However, their efforts were vital and contributed in no small measure to the success of the over-all program.

As the program developed, the need for some central clearinghouse for the dissemination of the details of the various chemical operations, knowledge of which was continually forthcoming from the co-operating laboratories, became paramount. Likewise, a plan for the distribution of the available intermediates in such a fashion as would promote the greatest efficiency was vital. To accomplish these ends, such a clearinghouse was established at the office of the Secretary of the Panel on Synthesis. To this

office came a steady flow of chemical procedures, so detailed that a person reasonably trained in the art of organic chemistry could duplicate them. Periodically a bulletin of such procedures was issued. This bulletin was available to anyone who was connected with the program and to whom it might be of interest and use in facilitating the work. In addition, personnel from the Secretary's office were constantly on the road visiting the various laboratories, with the sole end in mind of placing at each contractor's disposal the cumulative experience of the group as a whole. In this manner much wasteful duplication of effort was avoided.

Throughout the program, one thought was foremost in the minds of the synthetic organic chemists. This was to produce a drug in the shortest possible time, regardless of the yields involved. The question of yields requires some explanation. By yield, to the synthetic organic chemist, is meant the percentage of the theoretical yield (always 100 per cent) of the reaction product resulting from the interaction of two reactants. Rarely is this optimum percentage achieved in practice, owing to the formation of undesired byproducts or for other reasons. Particularly, it is not achieved in the first trial of a given reaction; rather, the attainment of optimum conditions is the result of a long series of experiments during which one variable after another is successively studied until the over-all optimum conditions have been determined.

Such a detailed study necessarily requires time and the expenditure of many man-hours of trained scientific help, of which an acute shortage existed throughout the war. As a result, the normal peacetime standards of scientific research were compromised. When the synthesis of a given drug was indicated, the drug was prepared in quantities sufficient for preliminary testing without regard for optimum yield, waste of raw materials, or waste of manpower. Only after sufficient indication was obtained of the potential worth of the drug was attention given to improvements in its synthesis or to the concurrent questions of optimum yields or cheap starting materials. In other words, the primary object at the exploratory level was to produce the proposed compound, regardless of the cost, in sufficient amount to permit a preliminary appraisal of its effectiveness as a drug. If this turned out to be favorable, attention could then be directed toward improved methods of synthesis.

In the light of the foregoing comments, let us consider the history of a drug known as SN 7618, or chemically as 7-chloro-4(4-diethylamino-1-methylbutylamino) quinoline. This drug was first synthesized by the German I. G. Farbenindustrie during the 1930's. For various reasons, among which may be cited the failure of the German pharmacologists and clinicians to appraise the value of the drug as an effective antimalarial, its potentialities were never exploited in Germany. In addition, the synthesis of the basic intermediate for the drug was dismissed by the Germans as being too im-

practical for commercial production in competition with established anti-malarials. During the course of a systematic reinvestigation of various German drugs, the high efficiency of SN 7618 as a malarial suppressive was discovered by American pharmacologists and clinicians. Up to this point, the drug, and particularly the intermediate 4,7-dichloroquinoline, had been prepared on a laboratory scale by a method admittedly not capable of trans-lation into cheap commercial production. When the efficacy of the drug became apparent, efforts were directed to the development of an improved synthesis of 4,7-dichloroquinoline. The result was the discovery not only of one such synthesis on the laboratory scale, but of at least three syntheses.

With the discovery of satisfactory laboratory syntheses, the problem arose of translating the laboratory-scale operations into full manufacturing-scale production. Here the "know-how" of the American chemical industry came into play. On the basis of only two weeks' laboratory experience with the new synthesis, the Committee on Medical Research initiated a contract with one of the major chemical industries of this country to carry on development and manufacturing research. During the course of this development project, difficulties naturally made their appearance. To supplement the manpower available, several other investigators were called in to aid in solving particu-lar problems as they arose. Thus, through the whole-hearted co-operation of chemist, chemical engineer, pharmacologist, and clinician the potentialities of a drug that had been missed by the vaunted German drug industry were realized.

As another example of the ingenuity of the American chemist, the case of the chemical class of substances known as the 8-aminoquinolines may be cited. This field of organic chemistry had been thoroughly plowed by the German dye trust during the 1920's and 1930's in the hope of discovering useful antimalarials. At the start of our program, it was held that, although the 8-aminoquinoline group undoubtedly possessed high antimalarial prop-erties, the substances were also characterized by inherent toxicity too high to warrant their practical use. When the problem of either increasing activity or decreasing toxicity in this group was suggested to the synthetic chemists by their pharmacologic colleagues, they attacked it with energy. The result has been that not only have new, more active and less toxic antimalarials been discovered in the 8-aminoquinoline group, but evidence has been produced and substantiated that the synthetic methods used by the Germans led to a highly impure preparation of their most effective drug in this category, plasmochin (pamaquine). Methods have now been developed that enable the production of pure pamaquine and also of several of its more effective chemical relatives.

Throughout the course of the antimalarial program, it was the constant policy of the Panel on Synthesis not to concentrate its entire manpower on the synthesis of drugs for which some antimalarial activity could be pre-

dicted, either on the basis of new or of old knowledge. Rather, it was the aim to utilize as high a proportion as possible of the chemical manpower available, consistent with the immediate demands of the program as a whole, for exploratory research into chemical classes of compounds in which antimalarial activity had not been noted before or which had been discarded for one reason or another.

As might have been expected, most of the efforts expended along this line were fruitless. However, notable success has apparently attended research along two lines of attack. In the group of 8-aminoquinoline derivatives, workers in the past had devoted comparatively little attention to certain variations in the radical attached to the quinoline nucleus by replacement of the eighth hydrogen atom; rather, certain standard types of variations had been used. By careful analysis of past work, the missing variants have now been synthesized to a large extent by the application of novel synthetic methods. The result has been the discovery of several new drugs which, at the time this manuscript is written, give promise of superiority over existing ones.

Along the same lines, attention has been directed to the replacement of several of the other hydrogen atoms of the quinoline nucleus by various radicals, which had not been done heretofore. Here again, from present indications, the result has been new and more efficacious drugs.

As indicated previously, suggestive leads as to substances to be synthesized were also forthcoming from the results of studies of the action of vital organs on known antimalarial drugs. Such studies may take the form either of in vitro experiments, wherein isolated animal organs are allowed to act on drugs, or of in vivo experiments, wherein the drugs are fed to normal human beings and the degradation products of the drug are isolated from the urine or feces. As an example of the former class of experiments may be cited the experiment in which quinine, the oldest of the antimalarial drugs, was subjected to the action of rabbit liver. Quinine is a derivative of quinoline in which one of the hydrogen atoms, called by the chemist the 4-hydrogen atom, is replaced by an exceedingly complex radical. It was found that under the action of slices of rabbit liver, quinine was attacked in the 2-position of the quinoline portion of the molecule rather than in the complex radical in the 4-position. Similar results were obtained by a study of the degradation products of quinine isolated from the urine of volunteers.

The synthesis of the complicated quinine molecule, accomplished through a brilliant effort of American chemists during the war, offered little hope of providing a more effective drug, since it had already been established by our medical colleagues that quinacrine was in all respects a drug superior to quinine. However, the fundamental groundwork for taking advantage of the results of the studies of the degradation of quinine had already been laid by British investigators. In an effort to simplify the quinine molecule, and

hence to render practical the synthesis of a chemical relative of quinine, these workers had published the synthesis of a simplification of the quinine molecule, which while possessing about half the activity of quinine against avian malaria, at the same time showed only about half the toxicity of quinine. The net result was a drug of about the same efficacy as quinine.

The results of the studies of the degradation of quinine were now applied to the synthesis of simplified drugs following the English lead, and relatives of the quinine molecule have been synthesized in which the vulnerable 2-position of the fundamental quinoline system has been blocked by such substituents as will impede attack under in vivo or in vitro conditions. The result has been the synthesis of a number of drugs that are vastly superior to quinine in antimalarial action but still leave something to be desired insofar as toxic manifestations are concerned. It is one of the unfinished assignments of wartime research to produce a drug of what may be called this "blocked quinine analogue" type, which will combine the high antimalarial activity so far attained with decreased toxicity not yet attained. Such a goal is not beyond the reach of postwar research.

Another general field in which useful antimalarial drugs may be found is that of the vitamins or their close chemical relatives. The idea that a minor variation in the chemical constitution of one of the vitamins essential to human existence might produce a substance that would not affect the metabolism of the host of the malaria parasite, but would deleteriously affect the metabolism of the parasite, has long been attractive. Despite the synthesis of many variants of components of the B-vitamin complex, it was only as the war program was drawing to a close that the first hopeful lead along this line came to light. A derivative of pantothenic acid, one of the constituents of the B-vitamin complex, has shown marked activity against avian malaria. Similarly, in earlier work certain substances related to vitamin K have shown antimalarial action. Whether this approach to the problem presents a useful point of departure must await the results of further work.

The road to the synthesis of new effective antimalarial drugs is not easy. Out of the fourteen thousand drugs tested during the wartime program, possibly ten hold sufficient promise to warrant high hopes for the benefit of mankind. One of the most valuable results of the work has been the stimulation of interest of many chemists in the problem. Many loose ends have been left hanging, and many suggested unexploited leads were abandoned at the termination of the war. It is one of the ironies of our civilization that it required the most terrible of all wars to set in motion the most concentrated attack in history on what is perhaps the most devastating of the infectious diseases of man. It is to be sincerely hoped that the impetus thus provided will not be allowed to die.

THE BIOLOGY AND BIOCHEMISTRY OF THE MALARIAL PARASITES

WILLIAM H. TALIAFERRO

THE BIOLOGY and biochemistry of the malarial parasites are of great importance, but they occupied a supporting and secondary position in the wartime program of malaria research. Their subsidiary nature followed from the fact that the primary aim of the work was the control and treatment of human malaria with drugs.

The achievements of the work on biology and biochemistry will be considered under five main headings, as follows: life-cycle studies; the maintenance, description, and standardization of various malarias; immunologic studies, including those bearing on the correlation of such studies with chemotherapy, artificial immunization, and serologic methods of diagnosis; biochemical studies, including the cultivation of the parasite outside the body, the biochemical relations of the parasite to the host as indicated by nutritional requirements, and the main metabolic pathways of the parasite and probable disturbances in metabolism associated with the action of antimalarial drugs; and general aspects, including an evaluation of the main achievements.

The biology of malaria also logically includes malaria control by mosquito reduction and mosquito repellents. This subject is covered in Part Six.

LIFE-CYCLE STUDIES

Studies on the life cycle of the parasite in the vertebrate host are considered first because they are fundamental to the rest of the biologic work and serve as an introduction to it.

All true malarial parasites belong to the genus Plasmodium. The discovery of the plasmodium in the blood of human beings by Laveran in 1880 and the description of its development in the blood by Golgi (1886–1894) laid the foundation for our present knowledge of the part of the life cycle that occurs in the vertebrate host. Similarly, the discovery of the transmission of the parasite by mosquitoes by Ross in 1898 and the detailed studies of Grassi and his associates (1898–1899) formed a basis for our knowledge of development of the parasite in the mosquito. From these and subsequent

investigations, it was known at the beginning of the war that infected mosquitoes inject infective malarial sporozoites, and that parasites appear in the blood in the red cells (erythrocytic stages) after an incubation period, during which no parasites can be found. In the red cells, they either grow and divide and, after rupturing the cells, infect new red cells (asexual cycle) or grow into gametocytes (sexual cycle), which may be taken up by and infect a mosquito. The asexual cycle is usually synchronous. Accordingly, at any one time all the organisms are in the same stage of development, and when they periodically break out of red cells, they give rise in man and monkeys to characteristic periodic symptoms of chills and fever.

What was not known was the extent to which malarial parasites develop in cells other than the red cells (exoerythrocytic stages). The question of exoerythrocytic development as contrasted to erythrocytic development falls into two parts. In the first place, after the mosquito injects the infective stage or sporozoite, does the latter immediately penetrate red cells and start the erythrocytic cycle in the blood? This part of the question seemed answered when, in 1902, the German protozoologist Schaudinn described the entry of sporozoites into red cells and their direct initiation of the blood cycle of the parasite. More recently, however, this began to be doubted because, in experimental infections of animals, various investigators found a so-called "negative phase" immediately following the injection of sporozoites, during which the blood was not infective but the tissues were infective — that is, produced infection when injected into susceptible animals. The stages during this negative phase are now known as pre-erythrocytic stages. With the conclusive demonstration that antimalarials are highly active on blood stages but do not prevent infection, it has been generally assumed that they are inadequate against the sporozoite or pre-erythrocytic stages.

In the second place, do exoerythrocytic stages persist after the blood infection has been established? Shortly after the discovery of the malarial parasite, a few investigators postulated resistant exoerythrocytic forms to explain the difficulty of completely eradicating infections with drugs and to explain how the parasite persisted during long periods when the disease was latent. Most investigators were highly skeptical about these exoerythrocytic forms until about ten years ago, when they were demonstrated in a number of avian infections. Such forms have not yet been unequivocally demonstrated in man, although they have been postulated. These phases of the malaria cycle were therefore pre-eminently important.

Studies conducted under an OSRD(CMR) contract have elucidated in detail the development of the chicken parasite, *Plasmodium gallinaceum,* from the time it enters the vertebrate host until it reaches the blood. The success of this work can be credited to the fact that *P. gallinaceum* was made available and that sufficient funds were at hand to obtain large quantities of mosquito glands infected with sporozoites. These studies also disclosed

information on the pre-erythrocytic development of several plasmodia in the pigeon and canary, and on the developmental series in *P. lophurae* in the turkey, duck, chicken, and guinea fowl.

Some remarkable facts about pre-erythrocytic stages have been uncovered. Sulfadiazine, which is prophylactic in that it apparently completely prevents infection of *P. gallinaceum* in the chicken, does not kill sporozoites but destroys certain of the pre-erythrocytic stages. Immunity of birds to erythrocytic stages is not necessarily associated with immunity to pre-erythrocytic stages. Thus, on the one hand, the canary is completely immune to both pre-erythrocytic and erythrocytic stages and, on the other hand, the chicken is susceptible to both stages. Between these extremes, the goose is highly susceptible to pre-erythrocytic forms but only slightly susceptible to erythrocytic forms, whereas the duck is also highly susceptible to pre-erythrocytic forms but almost completely immune to erythrocytic forms. Furthermore, after chickens have acquired a high degree of immunity to the erythrocytic infection by recovery from repeated attacks, they are still susceptible to pre-erythrocytic stages. Similar results have been obtained with other avian malarias.

With such success in uncovering the pre-erythrocytic development of avian parasites, it was hoped that homologous stages in the human infection could be found with comparative ease. In spite of the expenditure of enormous amounts of time and energy, however, this part of the problem has remained unsolved. It seems most probable, however, that the human parasite develops in different sites than does *P. gallinaceum*.

It was further found that pre-erythrocytic stages of *P. gallinaceum* developing from the sporozoite eventually become exactly similar to the persisting exoerythrocytic stages originally found by British investigators. This work indicates that exoerythrocytic stages occur in the chicken from the time of the introduction of the sporozoite throughout the entire infection.

Before the initiation of this program, investigators had been unable to find any exoerythrocytic stages of *P. cathemerium* in infections that had been passed from canary to canary by blood over a long number of years, but found them later in infections derived from the mosquito. The question then arose whether exoerythrocytic forms are characteristic of sporozoite-induced infections and not of certain blood-induced infections. Investigators found persistent exoerythrocytic stages in sporozoite-induced infections of *P. lophurae* in canaries, turkeys, pheasants, and zebra finches, but only once in ducks and not at all in chickens. Although the failure to find such forms in chickens and in most ducks may have been due to the presence of low-grade infections, it is significant that they have never been encountered in any blood-induced infections, which are frequently very intense.

It has been possible to follow the general severity of exoerythrocytic stages in the brain of chickens infected with *P. gallinaceum* by an ingenious method

of brain biopsy. By removing small samples of brain tissue from the cortex of the cerebrum, smearing them on microscopic slides, and staining them, the brain of the same chicken can be studied throughout the major part of the infection without any obvious deleterious effects on the chicken. It appears that quinacrine and quinine, which have no prophylactic action on this infection, have no observable deleterious effect on the exoerythrocytic stages, whereas sulfadiazine, which has a high prophylactic activity, damages them and eventually causes them to disappear from the brain. Approaching the problem from another angle, investigators studied the effect of drugs on malarial parasites in tissue culture using chick embryos infected by both sporozoites and blood stages of *P. gallinaceum*. In attempting to discover a test for the viability of exoerythrocytic stages that had been acted on in vitro by drugs, the investigators found that such forms set up infections in chick embryos when placed on the chorioallantoic membrane. The full potentialities of this interesting technic have not been explored.

THE MAINTENANCE, DESCRIPTION, AND STANDARD-IZATION OF VARIOUS MALARIAS

Thousands of chemical compounds of all kinds were subjected, as has been noted before, to extensive screening tests in experimental infections, mostly in birds. Also, restricted chemotherapeutic tests were done with two simian infections. To standardize the various malarias for pharmacologic tests and to facilitate biologic and biochemical problems, these infections were studied in detail. Moreover, these malarias were studied in various hosts, and other hosts were sought.

In using animals for screening antimalarials, serious difficulties arise because of the strict adaptation of the human parasites to man and the peculiar distribution of the other malarial parasites in animals. Thus, the parasites of man cannot be transmitted to an easily available laboratory animal, and most other plasmodia occur in birds, with only a few in reptiles and in mammals. Moreover, the mammalian forms, except for a few in exotic animals, are found exclusively in primates. Therefore, the only chance of getting a parasite similar to the human parasite, in a host, whose general metabolism is probably similar to that of man is to use simian parasites. But monkeys are expensive and difficult to handle. As a consequence, although the whole physiology of the avian parasite, as well as the host metabolism of drugs, is probably different from that of man, most of the preliminary screening had to be done with avian parasites.

Screening of malarial drugs prior to the war was done on *P. relictum* and *P. cathemerium*, which occur naturally in passerine birds, such as the English sparrow, and can be maintained in the laboratory in canaries. The antimalarial properties of the famous German synthetics, quinacrine and pama-

quine, were discovered with *P. relictum* in canaries. Supplementary studies on the gametocidal activity of drugs were also made with a relative of the true malarial parasites, Haemoproteus, of Java sparrows because only gametocytes of this parasite occur in the blood cells.

For years, workers had dreamed of a cheaper, sturdier animal than the canary on which to work. This dream was partially realized when *P. galli-naceum* was rediscovered in domestic chickens from Ceylon and introduced into European laboratories by the French parasitologist Brumpt, in 1935. Unfortunately, however, since the parasite produces a fatal disease in chickens and can be transmitted by all species of mosquitoes belonging to the ubiquitous genus Aedes, its introduction for scientific work into this country was rightly prohibited by the United States Department of Agriculture. Subsequently, in 1938, Coggeshall, then of the Rockefeller Foundation, found a parasite in a Borneo fireback pheasant, which he named *P. lophurae* and which he succeeded in establishing in the chicken. In 1940, Wolfson at Johns Hopkins University found that this parasite could be transmitted to ordinary ducks and would produce much heavier infections than in the chicken. In selecting suitable screening tests, it was generally agreed that *P. cathemerium* and *P. relictum* in the canary could be replaced by *P. lophurae* in the more readily available and less expensive duck. This infection, transferred by blood, played an important part in screening throughout the program. The proper authorities were requested to permit the introduction of *P. gallinaceum* into this country as a war emergency. This parasite furnished both blood- and sporozoite-induced infections for much of the screening as well as general biologic work. Its escape was elaborately guarded against and, so far as is known, successfully prevented. Although a number of drugs were tested on *P. knowlesi* in the rhesus monkey, which had been isolated from a related monkey of India, the crab-eating macaque, this parasite was later found unsuitable for drug testing because infections with it behave differently from the human infections toward a number of drugs. Blood-induced infections with *P. knowlesi* have, however, been used in biologic and biochemical work. Finally, during the latter part of the program, *P. cynomolgi,* in the rhesus monkey, which was also isolated from the crab-eating macaque, was intensively used in both blood- and sporozoite-induced infections.

Largely as a result of standardizing certain strains for pharmacologic tests, biologic data of great significance have been obtained. Thus, blood-induced *P. lophurae* has been studied in ducks and chickens, and *P. cathe-merium* in the canary; also, the differential effect of quinine, quinacrine, and pamaquine on the cell structures of *P. lophurae* has been investigated.

P. gallinaceum in chickens has been studied from many standpoints, including statistical data on chickens infected with both blood stages and sporozoites, with respect to the course of the blood infection; the occurrence

of exoerythrocytic stages and the mortality; the course of the infection and the asexual cycle of the parasite; various aspects of the pathology of the infection; and methods of handling infected mosquitoes, obtaining standardized suspensions of sporozoites, and artificially introducing them into chickens.

Among the simian parasites, *P. knowlesi* has been extensively studied, and a concerted effort has been made to standardize sporozoite-induced infections of *P. cynomolgi* in monkeys. This led to an intensive examination of the course of the infection of both blood-induced and sporozoite-induced infections.

The clinical investigators under OSRD(CMR) contracts, together with those of other governmental agencies and the British investigators, have assembled a unique mass of data on human infections. At the beginning of CMR work, there were four well-known domestic strains of human malaria that had been used for experimental work: the McCoy and St. Elizabeth strains of *P. vivax,* the U.S.P.H.S. strain of *P. malariae,* and the McLendon strain of *P. falciparum.* To these were added a highly virulent, frequently relapsing strain of *P. vivax* known as the Chesson strain, from the southwest Pacific, and a strain of *P. falciparum* from Costa Rica known as the Costa strain. Careful clinical studies of these strains with standardized doses of blood stages or of sporozoites were made in regard to the length of the blood infection, the frequency of relapse, various immunity reactions, and the reaction to drugs. These investigations have proved the individuality of the different strains and have made it possible for workers to obtain far more reproducible results than can be secured with the usual run of infections encountered at random in tropical hospitals.

The search for more infections that could be adapted to laboratory animals was continued.

Because success would have been so valuable, and even in the face of previous failures, systematic attempts were made to infect swine, sheep, kittens, rabbits, hamsters, guinea pigs, rats, mice, and chick embryos with various human parasites. The best survivals were in splenectomized mice made protein-deficient and in chick embryos, but a true colonization of the parasites was not attained.

The low-grade human infections present an acute problem when large numbers of parasites are needed for such work as is involved in immunologic and biochemical problems. Previous to this program, concentration of parasites was chiefly carried out by centrifuging blood, whereupon large parasitized red cells tended to collect on top of the rest of the red cells. The disadvantage of this procedure is that only large parasites can be recovered and many of them are injured.

One group of investigators effected the separation of the parasites by layering blood on a solution of plasma albumin of a critical specific gravity. After

centrifugation, uninfected red cells tend to fall to the bottom and parasitized red cells tend to remain suspended. Parasites obtained by this procedure are relatively normal.

The parasites were also concentrated by a method based on the differential movement of pigmented parasites in a powerful electromagnetic field. Other methods used include centrifugation for preparing highly concentrated and purified antigens of *P. vivax*. By lysing the red cells and subjecting the residues to differential centrifugation, a quantitative concentration of the parasites largely free of serum, leukocytes, and red-cell material has been attained.

IMMUNOLOGIC STUDIES

Prior to the war, the work of many investigators had shown that the defense of the body against malaria depends largely on the phagocytosis of parasites and parasitized red cells by the macrophages (related to the white cells) of the spleen, liver, and bone marrow. Furthermore, in some infections acquired immunity was found to be associated with a protective antibody.

It was discovered that serum from ducks recovering from several inoculations of *P. lophurae* gives slight and inconsistent protection against the infection in normal ducks. It was generally believed that such antibodies act as opsonins, which specifically make the parasites and parasitized erythrocytes more readily phagocytosed (engulfed and digested by the macrophages), although this had not been clearly demonstrated. With use of serum from chickens hyperimmunized to *P. gallinaceum* and *P. lophurae* and macrophages grown in tissue cultures, these opsonins were demonstrated. One unexpected aspect of this work was the finding that these opsonins are largely if not completely isoantibodies against the chicken red cell. The antibodies are similar to the isoantibodies of man, which are so important in blood transfusions, but instead of being natural they appear in chickens because of the repeated injection of red cells from other chickens during the process of hyperimmunization. Some of the immunity in malaria, therefore, may be a reaction to the red cell surrounding the parasite rather than to the parasite itself.

Among other general immunologic investigations was a study of the relation between the two malarias of chickens, *P. gallinaceum* and *P. lophurae*. Thus, when one infection was allowed to subside and the other was injected, recovery from *P. gallinaceum* was followed by a strong immunity to itself and to *P. lophurae,* whereas recovery from *P. lophurae* was followed by an immunity to itself but only a slight immunity to *P. gallinaceum*. Somewhat similar studies on immunity to superinfection were carried out during the standardization of several of the human strains of malaria.

Studying the effect of irradiating infected chickens, it was found that large doses of x-rays (500 to 600 r) frequently broke down the immunity of

chickens to both *P. gallinaceum* and *P. lophurae*. This is not surprising in view of the fact that the lymphocyte is one of the most radiosensitive cells of the body, that it may be involved in the formation of antibodies, and that it is an important source of macrophages.

IMMUNITY IN RELATION TO CHEMOTHERAPY

In view of the fact that the spleen is the most important organ in malarial defense, probably because it contains so many macrophages oriented to take material from the blood stream, and in view of the further fact that removal of the spleen decreases the efficacy of various chemotherapeutic agents, the relation of this organ to chemotherapy in chickens infected with *P. gallinaceum* was investigated. These studies revealed that, as with other infections, heavier blood infections and more fatalities occur in infected animals treated with quinine and splenectomized than in animals similarly infected and treated but not splenectomized. This decrease in the effectiveness of quinine in chickens without spleens is not due to a lower quinine level in the blood, because the levels obtained after a standard dose of quinine in chickens without spleens is higher than in chickens with spleens.

A series of experiments indicated that the following three factors play a role when chickens are treated with quinine: an innate immunity to the infection, which the chicken possesses before it has had any contact with malaria; an acquired immunity, which develops rapidly in the chicken after malaria is introduced; and a direct action of quinine on the parasite. Furthermore, the experiments indicate that removal of the spleen primarily affects the degree of acquired immunity. In fact, lowered acquired immunity more than cancels out any increased chemotherapeutic action that might result from the higher quinine blood level when the spleen is removed.

ARTIFICIAL IMMUNIZATION

In spite of a considerable body of knowledge regarding the immunologic mechanisms whereby the body recovers from malaria, comparatively little has been accomplished in artificial immunization against the disease with dead vaccines, and this has been done solely with the avian and simian parasites.

In order to answer the important question whether it is possible to immunize human subjects against malaria, vaccines were prepared from killed *P. vivax* parasites. This material was then injected serially into volunteers, who were later infected with either blood stages or sporozoites of *P. vivax*. It was also given to patients suffering with recurring relapses of *vivax* malaria in the hope that it would strengthen their immune mechanism and lessen the number of relapses. Unfortunately, none of these attempts were successful. The vaccines, despite their potency in terms of the number of

parasites used and the amount of material injected, had no effect on the prevention, delay, or course of the disease. The investigations are of great importance, however, because they are so well documented and controlled that they will serve as a useful guide to future workers, and because they have answered conclusively a question repeatedly raised in medical as well as lay circles concerning the feasibility of immunizing troops before they are exposed to malaria.

Serologic Methods of Diagnosis

For many years, the widespread use of such serologic methods of diagnosis as complement fixation was handicapped by the difficulty of obtaining large quantities of malarial parasites from which to make antigens. This difficulty was obviated by the discovery that an antigen reactive with serum from human patients could be made from *P. knowlesi,* which produces overwhelming infections. In addition, methods have been perfected to concentrate parasites from low-grade human infections, as previously described. The studies on serologic reactions as aids in the diagnosis of latent malaria gave useful negative information but very poor positive correlation. They did, however, throw additional light on some of the complex factors involved in serologic reactions in general.

BIOCHEMICAL STUDIES

Cultivation of Malarial Parasites

In 1911 and 1912, Bass and Johns of Tulane University first reported the growth of the erythrocytic forms of two species of human parasites. The work prior to the program of the Committee on Medical Research culminated in the studies of Trager, of the Rockefeller Institute for Medical Research, who got the erythrocytic stages of *P. lophurae* to survive for two weeks, but since he usually observed an increase in number of the parasites only during the first few days, they must have been dying more rapidly than they multiplied during most of this period.

Under the auspices of the Committee on Medical Research, the original technic of Bass and Johns was modified by placing plasma enriched with glucose in small, flat-bottomed test tubes with a thin layer of parasitized red cells on the bottom at 39.5° C. and in an atmosphere of 5 per cent carbon dioxide and 95 per cent air. Under such conditions, *P. falciparum* grows and segments in forty-eight hours, but does not invade new red cells and hence does not increase in number. The gametocytes, however, develop for seven to ten days, at the end of which time they appear to be similar to those in the blood of patients.

Outstanding advances in the method of cultivating parasites were made using *P. knowlesi, P. lophurae, P. vivax,* and *P. malariae.* Besides normal growth and segmentation, a marked increase in the number of parasites due to the invasion of new red cells, with subsequent growth and multiplication, has been obtained. The waste products of the parasites are either diluted or removed by diffusion through a semipermeable membrane. The suspending medium is complex and contains *p*-aminobenzoic acid as an essential component. Purified human serum albumin has been substituted for the plasma of the medium, and further work may disclose the essential nature of various other components of the medium.

METABOLISM

Metabolic studies of plasmodia were initiated in 1938 by Christophers and Fulton of England, using the simian parasite, *P. knowlesi.* These workers, as well as Maier and Coggeshall of the Rockefeller Foundation, Wendel of the University of Tennessee, with the support of the Tennessee Valley Authority, and Velick of Johns Hopkins University, showed that it was possible to study in vitro the biochemical properties of suspensions of red cells parasitized with various species of plasmodium. The aerobic utilization of glucose and other substrates by the parasite and the formation and accumulation of lactic acid as an intermediate in glucose oxidation were noted.

In 1941, as part of the OSRD(CMR) program, support was given to biochemical studies on *P. knowlesi, P. lophurae,* and *P. gallinaceum.* This action was based on the expectation that the effect of the antimalarials must ultimately be explained in terms of participation or interference with one or more of the metabolic reactions of the parasite. One group studied the effect of antimalarial drugs on the metabolism of the parasite, particularly the oxygen uptake. Another studied the carbohydrate and nitrogen metabolism of the erythrocytic form of *P. gallinaceum,* both within and free of the red cell, the effects of x-rays on the metabolic activity of the parasite, and the factors involved in the distribution of quinine between parasitized erythrocytes and the external medium. A third group used in vitro cultivation for studying the metabolism of the parasite and the action of antimalarial drugs on the growth of the parasite. A fourth group began its program by examining the physical constants of various antimalarials in relation to activity and the effect of antimalarials on isolated enzyme systems. Their later work involved experiments on the metabolism of erythrocyte-free parasites and on the effect of antimalarials on the metabolic reactions of the organism.

The present biochemical information is derived from the work of these four groups of investigators and, in addition, from data concerning the breakdown of nuclear protein by the parasite reported by Miller and Kozloff, of the Naval Medical Research Institute.

The available evidence, derived from the specific action of certain toxic

agents (cyanide, azide, diethyldithiocarbamate, and carbon monoxide), suggests that heavy-metal catalysts of the type already known as components of the Warburg-Keilin cytochrome system are involved in the respiratory mechanism of the parasite. The presence of flavoproteins and dehydrogenase systems, requiring diphosphopyridine nucleotide and triphosphopyridine nucleotide, as well as mechanisms for the synthesis and metabolic transformation of cholesterol and phospholipid, has been demonstrated, although the role of these substances in the economy of the parasite is unknown.

So far as nitrogen metabolism is concerned, the parasite seems capable of rapidly breaking down intracellular protein (hemoglobin or nucleoprotein or both) for its use in the formation of protoplasm. Proteolytic enzymes that digest protein have been obtained from the parasite in cell-free extracts. In the intact parasite, digestion of protein is apparently associated with aerobic metabolism, because the ability to break down intracellular protein is inhibited in proportion to the extent to which oxygen is excluded or its utilization is inhibited by antimalarial drugs. The proteins and amino acids of plasma also contribute to the nutrition of the malarial parasite.

The most detailed metabolic information concerns the carbohydrate metabolism of the parasite. Glucose is the principal carbohydrate foodstuff and is converted quantitatively into lactic acid under anaerobic conditions. From studies with cell-free enzyme extracts from parasites, the formation of lactic acid from glucose appears to involve a phosphorylating glycolysis similar to that occurring in muscle, liver, and other tissues of higher animals. In the presence of oxygen, the lactic acid formed from glucose is oxidized to carbon dioxide and water. The rate of formation of lactic acid from glucose is so rapid that it accumulates even under aerobic conditions. The oxidation of lactic acid to carbon dioxide and water proceeds through the intermediate formation of pyruvic acid, which is subsequently oxidized through a series of reactions similar to the tricarboxylic acid cycle involved in the carbohydrate oxidation in muscle of higher animals. With parasites liberated from the erythrocyte by the action of a specific hemolytic antiserum, pyruvic acid is in part oxidatively decarboxylated to acetic acid. The same reaction occurs in the intact parasitized erythrocyte to a physiologically insignificant extent.

Red cells parasitized with *P. knowlesi* consume approximately sixty times as much glucose and oxygen as do normal red cells. About half the glucose that disappears in the presence of parasitized cells is accounted for as lactate, and not more than one sixth of the glucose that disappears is completely oxidized. Various organic phosphate fractions and the flavine adenine dinucleotide content of the cell are also increased.

It is not yet possible to ascribe the action of any antimalarial to an effect on a specific metabolic reaction of the parasite. In general, the aerobic metabolism of the parasite is more sensitive to antimalarials than is the anaerobic metabolism, as ascertained with quinine and quinacrine. In the case of

quinine and quinacrine, pyruvate oxidation in *P. gallinaceum* is inhibited, while glucose utilization remains unimpaired. On the other hand, under certain conditions quinacrine primarily affects the hexokinase (glucose-phosphorylating) mechanism. In addition to the effect of drugs on carbohydrate metabolism, the breakdown of intracellular protein is inhibited by quinine and quinacrine.

All the metabolic reactions that have been described as occurring in the parasite have analogies in other tissues. The enzymes responsible for these effects, however, need not necessarily be the same. Observed differences in the sensitivity to antimalarial drugs between parasitic enzymes and those from other tissues suggest that the actual composition of the enzyme systems themselves may be different. A further study of these facts is necessary to understand the differential effect of chemotherapeutic agents on the parasite and its host.

The biochemical work has been concerned with the erythrocytic stage of the malarial parasite. With the development of new technics and a better knowledge of various stages, biochemical work may be extended to such stages. The data at hand, while admittedly fragmentary, represent the most complete information available concerning the enzymic composition and metabolic properties of a member of the protozoa.

Since malarial pigment is formed from hemoglobin, only parasites living in cells containing hemoglobin manufacture it. Accordingly, studies were conducted on the formation of malarial pigment or hematin by the parasite. Information on the action of quinacrine, quinine, and pamaquine on *P. knowlesi* was sought, and it is believed, on the basis of these studies, that the drugs may act by interfering with the enzyme system that the parasite uses to split hemoglobin.

In an entirely different way, evidence was found for the same conclusion in infection with *P. elongatum*. This parasite infects not only red cells but various precursors ranging in hemoglobin content from zero to that of the usual red cell. Quinine affects only the parasites in cells with hemoglobin, and hence only parasites with the power to form pigment.

SUMMARY

There is absolutely no question that the biologic and biochemical data obtained under the auspices of the Committee on Medical Research are of great scientific importance, not only in giving a better understanding of malaria and the malarial parasites but also in advancing the general subjects of infection, parasitism, and metabolism. Especially important have been an analysis of the major steps in the carbohydrate metabolism of several malarial parasites, new knowledge of the life cycle of several parasites in their vertebrate hosts, the quantitative description of a number of infections, and data

on certain aspects of immunology. Moreover, many of these results were essential in furthering the main program of finding chemotherapeutic drugs effective in man. Thus, all the data obtained on the biology of the human and animal malarias, including work on the life cycle of parasites, on the quantitative description of infections, and on immunology, aided in controlling and understanding the infections being used for chemotherapeutic tests and in interpreting the results.

CHAPTER L

THE SCREENING PROGRAM

GEORGE A. CARDEN, JR.

A S EMPHASIZED in the preceding chapter, many difficulties were encountered in the search for a suitable screening test for the vast number of compounds presented to the testing laboratories. The research that led to the discovery of the antimalarial properties of pamaquine and quinacrine was carried out on *relictum* malaria in the canary.

In these studies quantitative evaluation of antiplasmodial activity was attempted by making use of the chemotherapeutic index, a concept introduced by Ehrlich. This is the ratio of the maximum tolerated dose of the drug to its minimal effective dose. It has been considered a measure of antimalarial value. Unfortunately, chemotherapeutic indices determined on birds frequently bear no relation to those in human malaria and may be more misleading than helpful. It appeared to be important in future work to test for antimalarial activity in avian infections and to test for toxicity in mammals. One group of investigators, in studying the action of the cinchona alkaloids and their derivatives on malarial infections in the canary, expressed activity in figures representing the dose of quinine necessary to produce the same response as a unit dose of the alkaloid under test (the quinine equivalent). This point of view was a definite departure from the chemotherapeutic index measured in birds and used exclusively by the Germans.

A difficulty in the testing of drugs for antimalarial action on avian infections is that host and species of parasite are both different from those involved in the human diseases. In 1943, the literature on the activity of drugs in avian, simian, and human malarias was reviewed. Two conclusions may be drawn from this review. First, studies have been conducted in the past on all malarial infections in such a way that results of activity can be expressed only in qualitative terms such as no action, doubtful action, slight action, or pronounced or definite action. Second, the gaps in our knowledge at that time were so numerous that trustworthy conclusions were difficult or impossible to make.

Without quantitative evaluation of the antimalarial activity in avian infections there was no satisfactory way to judge the significance of tests in different avian infections or the relations of these tests to activity in the human malarias. Also, without such data there was no way of establishing

correlations between antimalarial activities and such chemical structural changes as may be introduced in an attempt to obtain the best of a series of compounds. For these and other reasons, the quinine equivalent was introduced as an expression of antimalarial activity for therapeutic tests.

Sulfadiazine and certain other sulfanilamide derivatives have been found to exert complete prophylactic action in *gallinaceum* malaria in the chick. Accordingly, the potencies of drugs as prophylactics in this type of malaria in the chick can be expressed in terms of sulfadiazine, and the sulfadiazine equivalent has been introduced as an expression of antimalarial activity for prophylactic tests in this infection.

The fact that avian and human malarial infections differ markedly in their respective host-parasite relationships poses the question as to the value of screening tests with avian malarial infections for judging antimalarial activity in human malaria. Do these tests discard drugs that may be of value in human malaria, and do they indicate high antimalarial activity of drugs that have little or no value in human disease? In other words, just what do the avian malarial tests mean for the selection of compounds for trial in man? Only a partial answer to these questions can be given.

The antimalarial activity of a drug in an avian infection may differ markedly from that exhibited in a human infection for a number of reasons. Some of these are as follows: differences in susceptibility of different species of parasites; differences in absorption, excretion, or distribution that result in different blood concentrations of the drug in birds and in human beings; and degradation of the drug to an active product in the bird and not in man, or, *vice versa,* degradation to an inactive product in man and not in the bird.

Similarly, differences in antimalarial activity of a drug on different avian infections may be due to real differences in species susceptibility or to differences in metabolic processes in the hosts. Differences due to species may be determined by using various species in the same host under identical testing conditions; and *vice versa,* differences due to host may be studied by testing the same species in various hosts under identical testing conditions.

In Table I are given certain selected tests of drugs on three species of parasites in two avian hosts. An attempt has been made to give also the antimalarial activity of the drug in *vivax* malaria in man. Several important conclusions may be drawn from the data given in this table. A drug (SN 8323) may be completely inactive in *lophurae* malaria in both duck and chick but have the same order of activity as quinine in *gallinaceum* malaria in the chick, in *cathemerium* malaria in the duck, and in *vivax* malaria in the human being. Here, the difference is dependent on species of parasite and not on host. Other examples of differences in the susceptibility of species of parasites are seen with drugs SN 112, SN 475, and SN 6865.

Differences in activity that are dependent on host rather than on species of parasite are seen with drugs SN 1452, SN 10,275, and SN 12,610. A host

Table I

Parasite and Host Differences in Response to Drugs (Therapeutic Test)
(Quinine equivalents)

SN*	Duck		Chick		Man
	P. lophurae	*P. cathemerium*	*P. lophurae*	*P. gallinaceum*	*P. vivax*
8323	< 0.15	1.0	0.10	2.0	0.5
7618	15.0	60.0	30.0	15.0	5.0
8137	3.0	6.0	30.0	20.0	4.0
971	60.0	150.0	40.0	10–80	10.0
1452	3.0	10.0	80.0	60.0	—
112	2.0	0.03	2.0	0.6	0.05
475	1.0	0.06	—	—	0.03
10275	20.0	80.0	2.0	2.0	1.0
6865	0.15	8.0	0.06	1.5	0.2
4271	2.0	1.0	1.0	0.6	0.2
12610	< 0.10	0.10	—	0.8	0.2

* SN 112 is *sulfadiazine;* SN 475 is *forbisen,* 2,2', 3,3'-tetramethyl-1,1'-diphenyl [4,4'-bi-3-pyrazoline]-5,5'-dione; SN 971 is *pamaquine, plasmochin,* 8-(4-diethylamino-1-methylbutylamino)-6-methoxy*quinoline;* SN 1452 is 8-(3-aminopropylamino)-6-methoxy-quinoline; SN 4271 is dimethyldithio*carbamic acid,* methylene diester; SN 6865 is 3,7-diacetamido-5-phenyl*phenazinium* ion; SN 7618 is 7-chloro-4-(4-diethylamino-1-methyl-butylamino) *quinoline;* SN 8137 is 1-(7-chloro-4-quinolylamino)-3-diethylamino-2-propanol; SN 8323 is 1-(*p*-chlorophenyl)-2-[4-(2-diethylaminoethylamino)-6-methyl-2-pyrimidyl] *guanidine;* SN 10,275 is 6,8-dichloro-2-phenyl-α-2-piperidyl-*4-quinoline-methanol;* SN 12,610 is *d*-N-(2-benzoylethyl)-α,γ-dihydroxy-ß,ß-dimethyl*butyramide.*

difference may occur within a series of chemical compounds where only a side chain is changed. Thus, SN 7618 and SN 8137 differ only in that one has a 4-diethylamino-1-methylbutyl and the other a 3-diethylamino-2-hydroxypropyl side chain. SN 7618 is definitely the more active drug in the duck, but no difference between the activities of the two drugs can be detected in the chick. In man the drugs are of the same order of activity. A drug may have the order of activity of quinine, or greater, on two or three species of avian parasites and prove inactive in *vivax* malaria in man (see SN 6865 and SN 4271).

From the above data, the conclusion has been drawn that for proper screening in avian infections two or more species of parasites and hosts should be used. In addition, this is apparently necessary when one is working within a definite chemical series to find the best compounds for human trial. It is doubtful that compounds of real value in human malaria will be missed if two avian parasites and two avian hosts are used when screening for trophozoite activity of drugs. However, it is impossible to say that any

one avian parasite or any one avian host is preferable if only one screening test is to be used. In different chemical series, the correlation of activity between avian and human infections varies.

In our discussion of the screening program in avian malarias, we have been considering only antimalarial activity against trophozoites in blood-induced infections. One must consider antimalarial activity against other stages in the life cycle of the malarial parasite — sporozoites, cryptozoites (or early tissue stages), exoerythrocytic forms (or late tissue stages), and gametocytes.

Studies on sulfadiazine and on quinacrine may be mentioned to illustrate some of the difficulties encountered in the present program. Sulfadiazine is a causal prophylactic against *gallinaceum* malaria in the chick but cures neither a sporozoite-induced nor a blood-induced infection. It is not a causal prophylactic against *lophurae* malaria in the turkey or against *vivax* malaria in man. In *knowlesi* malaria in the monkey, it cures the infection. In *vivax* malaria, it has a low grade of activity and does not cure. In *falciparum* malaria, sulfadiazine is a true suppressive. Quinacrine is neither a causal prophylactic nor a cure in *gallinaceum* malaria in the chick but is effective in suppressing blood-induced infections. In *knowlesi* malaria, it is not a cure but is highly effective against trophozoites. Quinacrine is effective as a cure in blood-induced *vivax* malaria but not in sporozoite-induced infections. However, in *falciparum* malaria quinacrine cures both blood-induced and sporozoite-induced infections.

The discovery of a true causal prophylactic for human malaria — a drug that would eradicate some stage of the parasite before trophozoites are produced — would constitute a great advance in the chemotherapy of malaria. On the other hand, a drug that would resemble quinacrine in its antimalarial properties but in addition would cure *vivax* malaria would probably be of more value than a causal prophylactic that did not cure (for example, sulfadiazine in *gallinaceum* malaria of the chick).

Several drugs belonging to different chemical groups have been found to be causal prophylactics in avian infections. From the data available at present, however, there seems to be a total lack of correlation between prophylactic activity in avian infections and that in *vivax* malaria in man. In Table II are given data to illustrate this point.

An assessment of the potential curative value of a drug in human *vivax* malaria from experiments conducted on the malarias of animals cannot be made at present. A number of studies have been made on the curative effect of drugs in avian malarias and, to a lesser extent, in simian malarias, but the data at present available do not allow any conclusions as to the correlation of these studies with curative action in human *vivax* malaria.

On account of this lack of an experimental infection for the screening of drugs as potential curative agents for *vivax* malaria, the problem of the cure of this human infection has been much more difficult than that of finding

TABLE II

Prophylactic Tests in Avian and Human Malarias

SN*	P. gallinaceum in Chick	P. lophurae in Turkey	P. cathemerium in Canary	P. vivax in Man
112	+++	o	+	o
8605	+++	+	+	o
5949	+++	+	+	o
11437	+++	+++	+	o
971	o	+	+	+++

+++ = Complete protection.   + = Significant delay in appearance of trophozoites over controls.   o = No effect.

* SN 112 is *sulfadiazine;* SN 971 is *pamaquine, plasmochin,* 8-(4-diethylamino-1-methylbutylamino)-6-methoxy*quinoline;* SN 5949 is 2-hydroxy-3-(2-methyloctyl)-*1,4-naphthoquinone;* SN 8605 is N¹-(5-bromo-2-pyrimidyl) *sulfanilamide;* SN 11,437 is N¹-(5-chloro-2-pyrimidyl) *metanilamide.*

a drug superior to quinacrine. The plan adopted has been to test for curative property in *vivax* malaria one or more examples of each chemical group showing even slight antimalarial activity.

The discarding of the chemotherapeutic index in the bird necessitates some screening test for toxicity. The antimalarial value of a drug is dependent not only on its antimalarial activity but also on its toxicity for the host. Emphasis was placed on the use of mammals for toxicity studies because it may be presumed that the common laboratory mammals are closer to man than are birds with respect to some of the processes of absorption, degradation, excretion, and so forth. Since most new compounds are available in limited amounts, the preliminary toxicity tests in mammals are performed on small animals such as the mouse or the rat. The latter studies have in general correlated roughly with studies on the dog and the monkey and with such information as has been obtained on man. However, in the case of the 8-aminoquinolines, marked discrepancies between the relative toxicities of members of this group have been found when tested on the mouse, the rat, the dog, and the monkey.

Toxicity tests are carried out in a manner to simulate as closely as possible the schedule of dosage to be used in human therapy — curative, suppressive, or prophylactic. Obviously, a determination of the acute toxicity (effect resulting from a single dose of the drug) is of no value in assessing the toxicity of the drug for use as an antimalarial to be given over several days. Thus, the acute toxicity of quinine for mice is equal to, or slightly greater than, that of quinacrine. On the other hand, a determination of the

short-term chronic toxicity (seven-, ten-, or fourteen-day administration) of quinine and quinacrine on both mice and rats indicates clearly that quinacrine is more toxic than quinine. This checks with experience in the human being, since it is well established that, on the basis of dosage, quinine is less toxic than quinacrine. However, since quinacrine is many times more active than quinine, the ratio between toxicity and effectiveness is greatly in favor of quinacrine.

These preliminary toxicity tests on the mouse or the rat are done in such a way as to attempt to maintain a more or less constant concentration of drug in blood and tissues during the whole period of observation. The schedule of dosage necessary to accomplish this will, of course, depend on the rapidity of absorption, excretion, degradation, or any combination of these of the particular drug in question. With many drugs a single oral dose per day will not maintain a concentration of drug in the blood at a desirable level. Two or three equally spaced doses per day can be given, or the drug-diet method can be utilized.

Before a drug is tried in a preliminary way for its antimalarial action in man, additional pharmacologic studies in the larger laboratory animals are necessary. In most instances, dogs or monkeys or both have been utilized for these additional studies. The characteristic toxic symptoms of the drug are elicited by administration of single doses by mouth or parenterally. With a knowledge of the type of toxic symptoms exhibited by the drug, experiments are performed to determine the short-term chronic toxicity. Here, as in the case of experiments in mice and rats, an attempt is made to have the dosage schedule resemble that to be used in man. Also, some appropriate drug is used as a standard of reference.

If the preliminary trial of a drug in human malaria suggests that further exploration of its value is justified, additional and more detailed pharmacologic and toxicologic studies in animals are conducted. These may involve studies of long-term chronic toxicity, as well as more detailed studies of the preliminary type.

Methods for estimating the concentration of the drug in the blood and in the tissues are then devised, and an extensive study is made of the distribution of the compound in the animal body, of the rate of absorption, and of excretion. These studies are exceedingly important. If a compound is poorly absorbed from the gastrointestinal tract, its action is very likely to be erratic, since the degree of absorption of a given oral dose is uncertain. If a drug is excreted too rapidly, it may condense in the kidneys and cause serious trouble. The fate of the compound in the animal body is further determined from the standpoint of metabolism; that is, whether it is handled as an intact molecule in the body or is broken into several parts and disposed of segmentally by the cells of the liver or excreted through the kidney. The fate of the compound in the lower animal and in man is often

quite different, however, since the correlation between monkey and man is much closer. Monkeys were used extensively in these special pharmacologic studies on new compounds being prepared for human trial.

SUMMARY

Thus, a mass of data was collected by the screening laboratories on the biologic activity of over fourteen thousand compounds in at least one and often two or three avian malarias in different hosts, and occasionally against monkey malaria as well. Biologically active compounds were further studied for toxicity and for special pharmacology in which the fate of the compounds was investigated intensively in the animal body. This accumulated volume of data was indexed, codified, and reproduced for distribution by the Office of the Survey of Antimalarial Drugs. It has thrown new light in many dark corners in the field of chemotherapy and will bear a great deal of useful scrutiny in the future.

THE CLINICAL TESTING OF ANTIMALARIAL DRUGS

JAMES A. SHANNON

No PROBLEM in clinical medicine has received such intensive study as has malaria during the last four and a half years. As a result of this concerted effort, advances have been made toward an understanding of the biology of the malarial parasite in birds, in monkeys, and finally in man; toward a more complete understanding of the natural history of the common types of malaria in man; and toward new and effective means for combating the disease by chemotherapeutic methods.

It is well to emphasize again that the effort was a co-operative one in the true sense. The clinical laboratories working under OSRD(CMR) contracts apportioned their tasks to the best advantage of the program as a whole, exchanged data, and supplied one another with infected mosquitoes or samples of blood whenever the need arose. Through the Board for Co-ordination of Malarial Studies, and through the agency of the panels under the Board, these clinical units integrated their work with the pharmacologic testing laboratories and with the synthetic chemists by guiding their efforts to make such changes in the chemical configuration of the molecular structures as would reduce toxicity of the compounds in a given series and maintain or enhance their effectiveness.

The ultimate objective of the studies was a general one: the improvement of antimalarial therapy. It was appreciated that complete success in this endeavor would involve, first, an improvement in suppressive therapy; second, an improvement in the treatment of the clinical attack; third, the development of agents that would cure *falciparum* and *vivax* malaria at a well-tolerated dosage; and finally, but perhaps of lesser importance, the development of agents that would prevent the inception of these diseases.

THE NATURAL HISTORY OF MALARIA IN MAN

It would have been helpful at the beginning of the studies had the exact disease mechanisms that underlie the malarial infections in man been known. In 1941, information on the natural history of these diseases in the wholly susceptible person was meagre, but it was adequate to formulate a reasonable

working hypothesis. This hypothesis possessed two advantages: it was amenable to experimental examination, and it could serve as a basic for the design of therapeutic tests that could themselves throw light on the underlying disease mechanisms.

THE MOSQUITO-INDUCED INFECTION

It seemed necessary to assume that the plasmodium of each of the malarial infections undergoes serial changes in the course of its residence in the human being, with the establishment of at least three discrete phases of development.

The human malarias are acquired naturally when sporozoites are introduced into the body through the bites of infected Anopheline mosquitoes. This may be taken as the first form of the parasite concerned with the human infection. It was also known that, while malaria may be easily induced by the transfer of blood containing parasites, the blood of a naturally infected person is noninfectious for a period of some five to seven days following the deposition of sporozoites by the mosquito. During this period — that is, the primary tissue phase of the disease — it was assumed that the sporozoites, or the forms of parasites derived from them, reside in the body in sites other than the peripheral circulation and undergo a developmental cycle that produces a form of the parasite capable of invading the erythrocytes. This invasion initiates the second, or erythrocytic, phase of the disease. The latter form of the parasite grows, segments, and divides or sporulates in the erythrocyte, with the production of new parasites with similar potentialities. This part of the life history of the parasite is commonly called its asexual cycle. The process of multiplication of erythrocytic forms continues in the normal person, with a progressive increase in the number of parasites in the blood until a sufficient density is reached to precipitate the fever characteristic of the clinical attack.

The subsequent course of the disease is conditioned by the species of the plasmodium involved, by the presence or absence of natural or acquired immunity, and by whether or not an attempt is made to modify the course of the disease through the use of therapeutic agents. A discussion of the clinical characteristics of each of the malarias as affected by these variables is not germane to the present discussion. However, in the design of therapeutic tests it was necessary to consider the mechanisms that might be responsible for the recurrence of clinical activity following the use of therapeutic agents. Little was known of these mechanisms except that such recurrences take place in a systematic fashion in *vivax* malaria and, to a lesser extent, in *falciparum* and quartan malaria.

Some investigators believed that these recurrences were due, in *vivax* malaria, to the simple persistence of resistant erythrocytic forms of the plas-

modium. They postulated a disease mechanism for *vivax* malaria that now seems adequate for *falciparum* malaria, but with one addition. It was necessary to assume that the persistent erythrocytic forms were in locations within the body where, perhaps, they were not accessible to therapeutic agents. Others believed that, since it is reasonable to assume there is a primary tissue form of the parasite, it is equally reasonable to assume that a tissue form persists. The latter appeared to be the more attractive hypothesis. In this view, provided therapy is adequate to interrupt the asexual cycle in *vivax* malaria, the relapse is attributable to the potentialities of the persisting tissue forms to undergo periodic sporulation, with the release of new lines of parasites capable of invading and growing in the erythrocytes and precipitating other bouts of clinical activity as discrete episodes in the course of the disease. In this view, the sexual forms of the parasite — that is, the gametocytes — are assumed to arise from the trophozoites of the asexual cycle. However, these forms, although of epidemiologic significance, have no importance in conditioning the course of the infection. They will receive no further consideration in this summary.

It follows from the assumption of such mechanisms that one must accept the possibility of there being at least three types of antimalarial activity exclusive of that which might become manifest against the gametocytes. One of these might involve the sporozoite; another, the primary tissue forms; and a third, the erythrocytic forms of the plasmodium. Also, since a persisting tissue phase of the disease was considered a strong possibility in *vivax* malaria, there might be a fourth type of antimalarial activity that would become apparent in properly conducted studies.

The Blood-Induced Infections

The blood-induced type of infection is established by the intravenous inoculation of blood containing parasites obtained from a patient with an active infection. These parasites are capable of growth, segmentation, sporulation, and invasion of new erythrocytes, as in the naturally acquired infection. However, the disease is limited to the single erythrocytic phase. Consequently, when the asexual cycle is interrupted by adequate therapy, it is not characterized by the spontaneous recurrence of clinical activity. Such a simple disease mechanism has the advantage of offering the opportunity to study the characteristics of a single phase of the disease and a single type of antimalarial activity; that is, the suppressive type. Furthermore, since the mosquito-induced *vivax* or *falciparum* infection can only manifest itself clinically by the establishment of an erythrocytic phase, it seemed reasonable to require complete information on this aspect of each infection before proceeding to the study of the more complex mosquito-induced diseases.

The majority of the information collected on the suppressive antimalarial activity of drugs has been obtained by using the McCoy strain of *P. vivax*

in blood-induced infection. Consequently, this infection will be discussed in some detail. Only brief comment will be made on the other infections that have been used.

Malaria is induced in the standard blood-induced McCoy infections by the intravenous inoculation of blood containing five hundred thousand parasites into a wholly susceptible patient; that is, one who has never had a previous attack of malaria. The infected blood is derived from a patient with an active infection on the fourth or fifth day of fever in the *vivax* infections and at some time prior to this in the *falciparum* infections. For present purposes, only two manifestations of the disease need be given consideration. These are the fever and the parasitemia that are characteristic of the clinical attack.

Following the inoculation of parasites, there is an incubation period of six days in the McCoy strain of *P. vivax*, unaccompanied by clinical manifestations of the developing disease. Thereafter, a progressively increasing and fairly well-sustained fever is observed. This lasts for at least five days before the febrile pattern becomes intermittent and is characterized by well-defined malarial paroxysms. These occur daily during the early days of the infection but may become tertian in character at a later stage. After a variable period of time, the febrile paroxysms diminish in severity, the temperature eventually stabilizing in the normal range. The clinical manifestations of the disease are then said to have terminated spontaneously. It is of particular importance to note that a spontaneous termination of the disease due to the McCoy strain of *P. vivax* does not occur, as a rule, until about fourteen days after the first day of fever.

Parasites are usually demonstrable by thick film a day or two before the first day of fever. Thereafter, they increase progressively in number until about the seventh to tenth day of fever, when they reach a maximum usually of some two thousand to ten thousand parasites per cubic millimeter of blood. The maximum is not well sustained and there is a progressive decline in density, anticipating somewhat the decline in the intensity of the febrile paroxysms. However, with the complete loss of fever — that is, with the spontaneous termination of the disease — a sizable density of the parasites is usually maintained for a considerable period of time. During this latter stage of the disease, the density of the parasites may fluctuate somewhat, and mild febrile episodes are not uncommon.

Blood-induced *vivax* malarias due to other strains follow a similar course. They vary as to the density of the parasitemia attained, the severity of the febrile paroxysms, and the period of activity before spontaneous termination. None of these differences require special comment at this time.

Blood-induced *falciparum* malaria, as used in routine therapeutic tests, has shown marked differences from the equivalent *vivax* malaria, as was to be expected. This type of infection was used on a rather extensive scale, first with the McClendon strain of *P. falciparum* and later with the Costa strain.

These infections, as contrasted to the McCoy *vivax* malaria, are characterized by a fulminating course, which, if not altered by the administration of an active drug, may proceed rapidly to a fatal termination. The disease due to either the McClendon or the Costa strain has a more variable prepatent period, exhibits a marked tendency toward an excessive density in the parasitemia, and is characterized by the occurrence of sustained febrile paroxysms. Also, if the infection is well supported during the early stages and antimalarial therapy is withheld, the duration of clinical activity is usually shorter than with *vivax* malaria.

The Susceptibility of the Parasite to Chemotherapy

The results attained from well-controlled studies with quinine in the treatment of standardized infections indicate that the erythrocytic phase of *vivax* malaria is equally susceptible to quinine whether derived from a mosquito-induced infection or from the transfer of infected blood. Stated in more general terms, the erythrocytic form of the *vivax* parasite appear to have the same chemotherapeutic characteristics in the two situations. Consequently, the relapse, which is a fairly consistent feature of the mosquito-induced *vivax* infection but does not occur in the blood-induced infection, cannot be reasonably attributed to the persistence of some portion of the erythrocytic phase of the disease; rather, it must be due to some other form of the parasite not present in the blood-induced infection. This is assumed to be a form of the parasite that is believed to persist in the tissue but has never yet been seen.

Information concerning the potentialities of these tissue forms to release new lines of parasites capable of invading and growing in the erythrocytes can be gained from a study of the subsequent course of the mosquito-induced infection. The course of the disease (McCoy *vivax*) was followed closely for a period of twelve or more months in 18 patients. Each clinical attack was treated with more than sufficient quinine to interrupt the asexual cycle. It is apparent from these data that the initial relapse rate is reasonably high, being in excess of 60 per cent within the first year, and that, characteristically, the first relapse occurs during the eighth or ninth month following the initial infection. These data indicate a rather definite pattern for the infection. Very extensive data of this type have been collected on another domestic strain of *P. vivax,* the St. Elizabeth strain. In addition, investigators have demonstrated, by subinoculation procedures, that during the period of quiescence or latency, as is to be expected, the blood is completely free of an infectious agent. The belief that the persisting form of the parasite responsible for the relapse is in the tissue is considerably strengthened by these data, as is the initial premise concerning the over-all disease mechanism underlying the *vivax* infection.

The sequence of events in a typical infection due to the McCoy or St. Elizabeth strain of *P. vivax* is as follows:

The initial clinical attack is interrupted by quinine, but the underlying tissue phase of the disease persists, remaining quiescent so far as overt clinical manifestations are concerned for a period of some seven months, at the end of which time a second erythrocytic phase is established. The clinical activity usually seen at this time is undoubtedly due to the maturation of persisting tissue forms of the parasite, which require this period before they are capable of releasing new transitional forms of parasites with the potentiality of invading the blood stream. Should the disease process again be altered by the administration of quinine or another therapeutic agent, further relapses may be expected in varying numbers and at varying periods of time.

Examination of the detailed data derived from the experimental subjects used in these studies indicates that quinine has an antimalarial activity in *vivax* malaria that, at well-tolerated doses, is suppressive in type and is wholly limited to the erythrocytic forms of the parasite. Consequently, quinine, or a drug with similar characteristics, can be used to define the activity of the underlying tissue phase of the disease with some precision.

This technic has been applied in a preliminary fashion to a virulent strain of Southwest Pacific *vivax* malaria known as the Chesson strain. In its early stage the disease is similar in all characteristics to the McCoy or St. Elizabeth *vivax* disease, but there is no long latent period between the primary attack and the relapse. Rather, one observes repeated clinical activity occurring in many patients at relatively short intervals. These relapses may occur as early as seven days after the termination of quinine therapy, or a comparable period of time following the fall in plasma quinacrine concentration to an infective level. It was believed that this type of *vivax* malaria would be invaluable in the examination of drugs for curative action, since the occurrence of relapses in a short time would permit a tentative evaluation of the effectiveness of an agent for curative action without requiring the long-term follow-up necessary when a domestic strain of *P. vivax* is used to produce the infection.

Before the Chesson strain could be used with confidence in studies of this type, it was necessary to determine which of two possible mechanisms is responsible for the short-term renewals of clinical activity. Such recurrence could be due to the normal relapse mechanism as described above for the McCoy *vivax* disease. In this view, the pattern of activity of the underlying tissue phase is such that it undergoes repeated short-time sporulations, each time releasing transitional forms of the parasite that are capable of invading the erythrocytes and precipitating a true relapse. It was also believed possible that the erythrocytic phase of the disease might be peculiarly resistant to the activity of antimalarials, and that the short-term renewals of clinical

activity were the result of an inability of the drugs used to interrupt the asexual cycle. This question was studied by examining the susceptibility of the erythrocytic phase of the disease to quinine and quinacrine in blood-induced Chesson malaria. The critical plasma quinine level required to terminate a standardized blood-induced infection is somewhat higher than for McCoy *vivax* and must be maintained for six, rather than four, days. Similar data are available on quinacrine. They indicate that the erythrocytic phase of Chesson *vivax* malaria is less susceptible to quinine and quinacrine than is that of McCoy *vivax* malaria.

This background of information on the natural history of *vivax* malaria permitted the design of therapeutic tests that made possible the examination of antimalarial agents for each of three types of activity; that is, the primary tissue phase of the disease, the erythrocytic phase, and the persisting tissue phase. As a convenience, these types of activity have been designated as prophylactic, suppressive, and curative, respectively.

Ideally, the study of the prophylactic action of a drug should be conducted in a manner whereby its therapeutic activity is limited to the first six or seven days of the infection. The persistence of a therapeutically active concentration beyond this time may be expected to alter the normal course of the infection through a suppressive effect on the erythrocytic forms of the parasite. This may be reflected in the absence of the initial clinical attack with domestic strains of *P. vivax* (McCoy or St. Elizabeth) or in the delay in the initial clinical attack with a strain having the characteristics of Chesson *vivax*. However, when a prophylactic test is interfered with in this manner by the persistence of a suppressive drug, other technics may be used to determine whether the suppressive has, in addition, a prophylactic action. Subinoculation procedures are invaluable for this purpose. For example, Australian investigators have demonstrated rather clearly that the currently recommended regimens of suppressive quinacrine therapy in no way delay the time of appearance of the erythrocytic forms of the parasite. Consequently, it may be concluded that quinacrine, in these doses, does not alter the initial phase of the disease and has no prophylactic action. Also, technics have been developed that permit the concentration of parasites contained in a 10-ml. sample of blood into as little as 10 cu. mm. of fluid. It has been shown, using a modification of this technic, that quinine and chloroquine (SN 7618), in full therapeutic doses, have no effect on the initial phases of the disease and have no prophylactic action.

Antimalarial activity of a suppressive nature being exerted against the erythrocytic forms of the parasite can be most simply evaluated through the use of a standard blood-induced infection, as previously described. Antimalarial activity of a curative nature can only be demonstrated in *vivax* malaria if therapeutic tests, which are performed in mosquito-induced infections, are conducted in a fashion that is known, on the basis of information derived from the blood-induced infection, to interrupt the asexual cycle of

the parasite, and if follow-up periods are sufficiently long to include a final estimation of relapse rates with the test drug as compared with the standard of reference.

Antimalarial activity of a curative nature in *falciparum* malaria can presumably be demonstrated in the blood-induced infection and is synonymous with antimalarial activity of a suppressive nature. This belief follows from the features of the disease indicating that there is no persistence of a tissue form of the parasite. Consequently, interruption of the asexual cycle of the erythrocytic phase, if accomplished subsequent to the completion of the initial episode of sporulation by the tissue forms, is all that is required for cure of the infection.

The discussion has so far been devoted largely to a consideration of the natural history of *vivax* and *falciparum* malaria, and of the disease mechanisms that underlie their clinical manifestations insofar as these condition the examination of substances for antimalarial activity. It is equally important to have an appreciation of certain fundamental concepts applicable to any situation wherein a chemical agent is used to produce a given therapeutic effect.

The activity of any chemotherapeutic agent must result from its ability to participate in or to interfere with some phase of biologic activity. However, the specific as well as the over-all activity of the agent will be conditioned by the factors that determine its ability to reach the specific site of its action, the concentration it achieves at that site, and the length of time a biologically active concentration is maintained. It follows from this that the examination of the chemotherapeutic action of a compound will be facilitated by the use of experimental technics that separate the factors directly related to the physiological disposition of the compound by the host from those directly related to the action of the compound in any given biologic system within the host.

For practical purposes, it has been assumed that this effect can be achieved in the malarias provided it is possible, first, to define the relationship between the concentration of an active agent in the plasma and its concurrent antimalarial activity, and second, to describe the operation of the processes that together determine the relationship attained between drug administration and the plasma drug concentrations of the active agent. Emphasis has been given to this view in the studies of antimalarials. This is partly because plasma is the medium of exchange of all substances as they are absorbed, distributed, and degraded in the body, processes that, together with other factors, determine their rates of renal excretion. Consequently, in any given situation the concentration of an antimalarial in the plasma may be taken as an integrated expression of the operation of these several processes. Furthermore, at least in the case of the erythrocytic phase of the disease, the concentration of an active substance in the plasma is that to which the parasitized erythrocytes are continuously exposed. The intelligent study of the

potentialities of any antimalarial agent therefore requires the availability of chemical methods to assay its concentration in biologic tissues and fluids and at times to follow its course of metabolism.

Examples have been uncovered of a complete lack of correlation between the apparent plasma concentration of a drug and its therapeutic effect. Two such examples warrant special comment.

The early study of the suppressive activity of one family of compounds, the naphthoquinones, in human *vivax* malaria was aided by a chemical method that depends on the development of a red color on the alkalization of an aqueous solution of the naphthoquinone extracted from biologic material. This is a general property of 2-alkyl-3-hydroxy-1,4-naphthoquinones. With this general procedure, there was a striking lack of correlation between the plasma drug concentrations observed and the therapeutic effects. This circumstance led to a careful study of the solubility characteristics of the naphthoquinones extracted from the biologic material and to the demonstration that the naphthoquinone contained in plasma was a mixture containing one or more degradation products. This information was useful for two purposes: it explained the lack of correlation observed, and, as with cinchonine, it constituted another example of the role that processes of degradation can play in the limitation of antimalarial activity. These findings were of importance in planning further synthetic work in this class of compounds.

The second example relates to the suppressive and curative activities of pamaquine. The therapeutic effects correlate reasonably well with the oral dosage of the drug, but not at all with the plasma pamaquine concentrations determined by a highly specific chemical method. These data, together with other information, suggest that such a circumstance arises from a situation wherein pamaquine itself is without a high order of antimalarial activity but produces its therapeutic effect incidental to the formation of a highly active metabolic product.

These and similar examples emphasize, rather than minimize, the importance of utilizing chemical procedures in the control of therapy. It is only with this approach that the vital factors controlling the physiological disposition of a drug may be examined.

THE STUDY OF DRUGS HAVING A SUPPRESSIVE TYPE OF ACTIVITY

Cinchona Alkaloids

It was first demonstrated that the early observers who studied the antimalarial activity of the various cinchona alkaloids (products of the cinchona tree, from which quinine is obtained) were essentially correct in their conclusion that cinchonine, cinchonidine, and quinidine each has an antimalarial

activity of a suppressive type comparing well with that which is possessed by quinine.

Earlier speculations concerning the mechanism of the antimalarial actions of the cinchona alkaloids included the possibility that these compounds have no direct action on the plasmodia. Similar suggestions have been made concerning the action of some of the antibacterials, but these have been found to be without basis when subjected to direct experimental examination. It seemed probable that a similar situation would obtain with the cinchona alkaloids, and that their antimalarial activity is derived from an ability to interfere with some biologic system within the parasitized erythrocyte that is essential for the growth and multiplication of the parasite.

An inquiry into the details of such an effect presupposes knowledge of the molecular species of at least one of the cinchona alkaloids capable of such an action. This need for information is highlighted by an appreciation of the marked differences in the antimalarial activities of each of the four alkaloids when the estimation is based on the plasma drug concentrations, as compared with the oral dosages required to produce a given therapeutic effect in the standard blood-induced infection. It seemed quite possible, in view of this circumstance, that, incidental to the more or less complete metabolic change of each of these structurally similar compounds, an intermediate substance or substances were formed with common chemical characteristics and that the latter were the active antimalarials. In such a situation, the amount of active material formed every twenty-four hours might be related more closely to the oral dosage of the alkaloid than to the plasma alkaloid concentrations attained.

Opposed to the acceptance of this hypothesis were the reasonably good correlation with each alkaloid between the plasma drug concentrations and its antimalarial effects, and the lack of a similarly good correlation with each alkaloid between oral dosage and antimalarial effects. However, the estimated difference between the antimalarial activities of quinine and cinchonine, when based on the plasma alkaloid concentrations, is so great that a search was made for more definitive evidence concerning the possible role of metabolism in conditioning the antimalarial activity of these compounds. Generally speaking, a metabolic change may influence the therapeutic activity of an agent in one of three ways. It may produce an active agent from an inert substance, as contained in the above hypothesis covering the cinchona alkaloids; this appears to be the case in the prontosil soluble-sulfanilamide system, and, as noted above, is possible for pamaquine. Second, it may limit the action of a substance through a chemical change that minimizes or removes the ability of a substance to exert a given action. Third, a substance may exert an action insofar as it enters a biologic system and exerts an effect incidental to the operation of the system resulting in a change in its chemical structure.

Cinchonine was selected for initial detailed study from among the cinchona alkaloids because its coefficient of metabolism is much higher than that of the other cinchona alkaloids and because, in contrast to quinine and quinidine, it has a simpler chemical structure. The main route of degradation of cinchonine in man appears to involve two serial steps. The first of these is an addition of oxygen to the quinoline nucleus with the formation of a carbostyril; the second is an addition of oxygen to the quinuclidine nucleus, perhaps at the vinyl group or the ring carbon to which the vinyl group is attached.

It appears that the oxidation of cinchonine to a carbostyril is due to the operation of an enzyme system contained in liver and other tissues, which produces a carbostyril from quinine. This enzyme system has not been isolated from human tissues, although its presence there may be assumed with confidence since the carbostyril has been isolated in large quantity from the urine of persons receiving cinchonine. The enzyme has been isolated from rabbit liver. It seems likely, then, that the antimalarial activity of cinchonine is due to one or another of these three molecular species or to a combination of the activities of all three. It was with this thought in mind that cinchonine was administered to patients so as to permit the isolation and purification of the first and second metabolic products, the former in sufficient quantity to study its antimalarial activity in blood-induced *vivax* malaria.

It appears likely from these studies that the antimalarial activity of cinchonine is derived from the ability of this substance, rather than an active intermediate, to participate in an essential biologic system of the parasite or a shared biologic system in the parasitized erythrocyte. By analogy, it would appear that this is also likely for the other cinchona alkaloids. This conclusion may serve as the basis for further studies concerning their mechanism of action.

It is to be emphasized that the importance of these observations on the antimalarial activity of the cinchona alkaloids does not stem from considerations of their use in the suppression and treatment of malaria. Rather, they are significant in that they define the general type of background information that must be obtained before an approach can be made toward an examination of the discrete biologic systems within the parasite, or parasitized erythrocyte, that are concerned with the antimalarial actions observed.

QUINACRINE

The second step that permitted the more effective use of antimalarial agents already available was the more practical one of the two. This was the acquisition of new information on the physiological disposition and antimalarial activity of quinacrine. It was tentatively assumed, and subsequently

established, that, similar to the cinchona alkaloids, the antimalarial activity of quinacrine is a reflection of its concurrent plasma drug concentration. Consequently, rational usage of the drug required information on the factors concerned with the regulation of the plasma quinacrine concentration on any regimen of therapy.

It was early demonstrated that, contrary to the cinchona alkaloids, effective plasma quinacrine concentrations are not rapidly achieved on any dosage regimen that administers the same-sized dose serially. This is because quinacrine is very extensively localized in many tissues of the body and is degraded at a low rate. Accordingly, when used as recommended in 1941 — that is, 0.1 gm. three times daily — therapeutically active concentrations are not usually obtained early in the course of treatment of the clinical attack. It was predictable from this fact that such therapy would not produce an abrupt termination of clinical activity, and, in fact, this is what had been found in practice. However, when large priming doses of quinacrine are given during the first twelve to sixteen hours of therapy, high plasma drug concentrations are quickly obtained and clinical activity is promptly terminated.

Similarly, it was found that the pattern of weekly dosage has little influence on the plasma quinacrine concentration maintained during a course of suppressive therapy. It was also found that when the same suppressive dosage is given to a number of normal persons, there is a very wide variation in the plasma drug concentrations achieved and maintained in the members of the group, and that when drug administration is limited to 0.4 gm. weekly, many persons in the group attain levels below those at which one would expect a suppressive effect.

It was possible, on the basis of these studies, to recommend a change in the dosage regimens for quinacrine when used as a suppressive and for the termination of a clinical attack. These recommendations were shortly adopted by the armed forces. One year's experience with what may be termed the rational use of quinacrine was sufficient to demonstrate that the drug is superior to quinine for the routine management of the malarias. Quinacrine will prevent the inception of clinical *falciparum* malaria when given as a suppressive and will effect a prompt and definitive cure when the infection is once established. However, it will not prevent the inception of *vivax* malaria, although it is highly effective in suppressing its clinical manifestations, and it will not effect a definitive cure when the infection is once established. It was not known until later that these two limitations are fundamental in character. The effectiveness of routine quinacrine therapy, however, was such that in the summer of 1943 it seemed likely that the clinical program could focus more of its attention on the specific problem of developing curative agents for *vivax* malaria. This marked the end of the first period of studies and the beginning of the second.

THE STUDY OF NEW COMPOUNDS HAVING A SUP-
PRESSIVE TYPE OF ANTIMALARIAL ACTIVITY

The major efforts of the next phases of the malaria program were de-
voted to the development of curative agents for *vivax* malaria.

It was believed that the development of an antimalarial with a high de-
gree of curative action against *vivax* malaria could be approached by one
of two means. One approach could be based on the hypothesis that the fun-
damental metabolic organization of the persisting tissue forms of *P. vivax*
is essentially the same as that of the erythrocytic forms of the plasmodium,
and that suppressive and curative activities in *vivax* malaria are the same.
Owing to a difference in its cellular environment, the tissue form could be
assumed to be less susceptible to the antimalarial effect of drugs such as
quinacrine. Accordingly, a reasonable approach to the development of cura-
tive agents appeared to lie in the direction of obtaining more active drugs
as evidenced by their ability to exert an action on the erythrocytic forms of
the parasite; that is, suppressive activity as manifested in the blood-induced
infection. It was hoped that if the intensity of this type of antimalarial activ-
ity was sufficiently great in the case of any drug, it would not only interrupt
the erythrocytic phase of the *vivax* parasites but would also exert a curative
action and obliterate the persisting tissue phase of a naturally acquired *vivax*
infection and so cure the disease.

The other approach could be based on the hypothesis that the fundamen-
tal metabolic organization of the persisting tissue forms of *P. vivax* is dif-
ferent from that of the erythrocytic forms of the plasmodium, at least insofar
as the susceptibilities of their essential biologic systems are concerned. Ac-
cordingly, a chemotherapeutic agent could affect the tissue forms through an
action qualitatively different from any that produces a dramatic effect on the
asexual forms in the erythrocyte. In accordance with this hypothesis, the
blood-induced infection might have little value in the search for curative
agents. Also, it was quite possible to miss a curative agent unless a number
of representatives of each group of chemicals studied were examined for
curative action in the mosquito-induced infection. Compounds could be
selected for such action because of special activities other than suppressive in
the avian infections, or they could be screened for curative action in human
vivax infection without prior experimental trial.

It was generally agreed that sufficient evidence was not available for one
to decide which of these two hypotheses was the more reasonable. Conse-
quently, there was some doubt whether blood-induced infections could be
of value in a program the end of which was the development of curative
agents for *vivax* malaria. However, this type of infection was continued in
use on a rather extensive scale, its employment being based on the tentative

acceptance of the reasonableness of the first working hypothesis. As a logical consequence and as the major effort at that time, a systematic attempt was made to increase the suppressive type of antimalarial activity in a number of the chemical series then under exploration. The best representative in each series was selected on the basis of information from blood-induced infections and examined for curative activity in mosquito-induced *vivax* malaria. Actually, the over-all procedure adopted represented a partial compromise between the two working hypotheses. Certain of the compounds studied for curative activity had relatively little suppressive activity, their selection being based on two considerations. They were representatives of chemical series as yet untried for curative action, and although they might have had little suppressive activity, the compound tested was better in this respect than the other members of the series examined. In addition, any compound showing a special type of activity in the avian infections, such as curative or prophylactic, was also tried for curative activity in *vivax* malaria.

The advantages of this approach, at that stage of the program, were three. First, it was believed likely, with the leads then available, that suppressive antimalarial activity could be increased many fold in several different types of compounds and that, as the result of this effort, compounds would shortly become available with which to test the correctness of the first working hypothesis. Second, this approach would permit the study of a number of chemical series, as yet unexamined, for their possession of curative activity and perhaps establish a correlation between special activities in avian infections and curative activity in *vivax* malaria. Third, it seemed reasonable to suppose that this approach to the problem would result in the development of antimalarials superior to quinacrine, although they might have the same fundamental limitations. The third possibility was important. It was desirable to have available antimalarials other than quinacrine, should the latter's long-term continuous administration to human subjects be accompanied by toxic manifestations that could not be predicted at the time. It was in line with the last thought that, although the systematic survey for the suppressive type of antimalarial activity was limited to studies in blood-induced McCoy *vivax* malaria, any drug with promise was also studied for effectiveness in *falciparum* malaria.

It was early demonstrated beyond doubt that the suppressive antimalarial activity of a compound, when measured in a single avian infection, may have little prediction value for the situation obtaining in the suppression of parasitemia in the human malarias. It was later shown that the sum total of the information, when derived from the study of the activity of a compound or series of compounds in several avian infections using several avian hosts, does have fair prediction value in the selection of compounds for trial as suppressives in the human malarias. Lastly, it was demonstrated, within

the compounds studied, that none had higher antimalarial activity of a suppressive nature in human infections, as compared to quinine, than had been observed in at least one of the avian infections. This information was accumulated incidental to the survey of a very large number of compounds (about fourteen thousand) for activity in the avian infections, of a limited number of compounds (about sixty-five) for suppression activity in the human infections, and of a selected number of the latter group (about twenty) for prophylactic or curative action or both in *vivax* malaria.

Of the newer suppressive compounds developed, the most promising are contained in the series of 4-aminoquinolines. About two hundred compounds of this group, with variations in nucleus as well as side chain, have been screened for antimalarial activity in at least one avian infection. A smaller number have been examined for toxicity in at least one species of mammal. About ten of these 4-aminoquinolines have been examined, first for toxicity in the dog and monkey and then for antimalarial activity and toxicity in man. Several of these ten appear to be superior to quinacrine. Chloroquine (SN 7618) has received the most extensive study in both civilian and military establishments. It is superior to quinacrine in a number of ways. Effective suppression can be obtained by administering it no more frequently than once weekly in a well-tolerated dose. It will cause an abrupt termination of the clinical attack of *vivax* malaria when used for only twenty-four to forty-eight hours, as compared to the usual five- to seven-day treatment with quinacrine. Furthermore, it will also cure *falciparum* malaria when administered for only one or two days. In addition, it does not stain the skin or produce gastrointestinal disturbances. Similar claims can probably be made for oxychloroquine (SN 8137), which has, however, had less extensive exploration. Other compounds still in the exploratory stage may have advantages over both chloroquine and oxychloroquine.

Another series of compounds that has been extensively studied, at least in experimental animals, comprises the quinolinemethanols. These compounds may be considered as being rather closely related to quinine. In 1938 two investigators reported the first compound of this series to be found active in avian malaria. This had the 2-piperidyl side chain. Later, two other investigators prepared a series of alpha-(dialkylaminomethyl)-quinolinemethanols and found them also to possess antimalarial activity. These two series have been familiarly called the A–K and K–W compounds. In the A–K series the original active compound was one quarter as active as quinine in *lophurae* malaria in the duck, whereas one of the most active compounds in the original K–W series was found to be one eighth as active as quinine. About two hundred compounds of the quinolinemethanol type have been prepared, and in both the A–K and K–W series we now have compounds about forty times as active as quinine. This is an enhancement of activity of two hundred to four hundred times that of the original compounds. About five compounds of this series have been tried in man for

antimalarial activity and toxicity, but owing to poor absorption or toxicity none of them have appeared promising enough for further trial. This series, however, is worthy of further study.

It was reported that the survival in vitro of *P. lophurae* was favored by the presence of calcium pantothenate. This observation resulted in the synthesis of about twenty-five analogues of pantothenic acid for antimalarial testing. In the first group of drugs synthesized, only one, α,γ-dihydroxy-$\beta,$-β-dimethyl-N-(2-sulfamylethyl) butyramide (SN 3259), was active, on the intravenous injection of very large doses, in *gallinaceum* malaria in the chick; it was, however, inactive in *lophurae* or *cathemerium* malaria in the duck. In addition, its activity in *gallinaceum* malaria was neutralized by administration of pantothenic acid. Subsequently, a compound synthesized by an investigator in association with this program was found, when given orally, to be as active as quinine in *gallinaceum* malaria in the chick, active in *lophurae* malaria in the chick, but inactive in *lophurae* malaria in the duck. This substance, pantothenophenone (SN 12,610), was also antagonized by pantothenic acid. In *vivax* malaria in man, pantothenophenone is definitely active. This is the first instance, so far as is known, in which an effective antimalarial agent has been found as a result of a logical rather than a hit-or-miss approach.

Before considering the third phase of the studies, it will be of some interest to take note of the potentialities of certain of the agents that, at least to some extent, are byproducts of an attempt to produce a curative agent for *vivax* malaria, with suppressive action as the criterion of effectiveness. The full potentialities of SN 7618, SN 8137, and several related compounds have not as yet been fully exploited. However, the information at hand permits the prediction that they will constitute a relatively simple means for the complete control of malaria in many areas, owing to the lessening of the administration problem of suppressive therapy as compared to quinacrine. They may also, in specific areas, contribute to the eradication of the malarias through their ability to curtail transmission of the disease. Exploration of the advantages to be derived from the use of some of these newer agents is now under way. Such studies must involve the comparative assay of the usefulness of the agents studied under conditions identical with those that will obtain during their routine use in the field.

THE STUDY OF NEW COMPOUNDS HAVING A CURATIVE TYPE OF ANTIMALARIAL ACTIVITY IN VIVAX MALARIA

The effectiveness of suppression by quinacrine, on the basis of the studies mentioned previously, accentuated the need for a curative drug. The success with suppressive therapy made it possible for more troops to be exposed to malaria without their becoming malaria casualties until after they had

been returned to the mainland and had stopped taking quinacrine. This enabled the Army and the Marine Corps to carry out clean-up operations in the malaria-infested islands of the Southwest Pacific rapidly and effectively. It was realized, however, that the time would come when these men would present a serious medical problem.

When the search among the suppressive type of compounds for a drug that would cure *vivax* malaria failed, it became necessary to reorient the thinking behind the entire program and to explore chemical substances with entirely different action from those studied to date. A serious obstacle to success in this endeavor was the complete absence of a satisfactory screening test. No curative tests in avian malarias had been devised, and there was considerable question whether such a test, if one should be available, would possess any significant prediction value in *vivax* malaria in man. Furthermore, prophylactic studies in avian infections with certain compounds, notably those related to the sulfonamides, had disclosed this type of activity. On the other hand, the sulfonamides showed no prophylactic action against *vivax* malaria.

The only lead that seemed worth following was uncovered by British investigators in the early 1930's. They showed that pamaquine, when administered in certain doses for a given period of time (fourteen days) concurrently with quinine, gave promise of curing *vivax* malaria.

The Committee on Medical Research investigators set about putting this thesis to experimental test. Specifically, the following questions were subjected to critical experimental evaluation:

(1) Does pamaquine alone have a unique action in influencing the subsequent course of the disease produced by a well-documented domestic strain of *vivax* malaria?

(2) Is this action prophylactic, curative, or both?

(3) Does quinine enhance the prophylactic or curative action of pamaquine, and if so, why?

When the results of these experiments were analyzed, the answer to the first question was undoubtedly "yes." The answer to the second is that pamaquine has both prophylactic and curative action. The answer to the third is also in the affirmative, without question, but the basis for this is not entirely understood. It can, however, be said with fair certainty that the action of quinine in enhancing the effect of pamaquine has nothing to do with its antimalarial action, since it acts only on trophozoites, which have no part in the production of relapses. From the experimental data available, it appears that quinine enhances the effect of pamaquine by affecting its metabolism in such a way as to augment its action against the tissue forms of the disease.

In the course of these studies on the unique prophylactic and curative action of pamaquine, the toxicity and general usefulness of this compound

was re-evaluated and the potentialities of related compounds began to receive intensive study.

It should be emphasized that the 8-aminoquinolines (pamaquine congeners) had received considerable thought early in the program and had been discarded for several reasons. First, the toxicity of pamaquine was known and feared on the basis, particularly, of a mass reaction that occurred in the course of a large study designed to test its effectiveness in controlling the spread of malaria in a native population in Panama. Second, it was known that pamaquine analogues had received systematic study by the Germans, the French, and the Russians, both before and after the development of pamaquine, and that no better drug had been found by these workers. Third, the toxicity of the analogues of pamaquine, which were studied by these investigators, appeared greater than pamaquine in most instances. Finally, as noted previously, it was hoped that a curative agent might be found in other series of chemical substances that would be relatively free from toxicity.

These essential facts relating to the antimalarial activity of pamaquine having been confirmed, it was demonstrated that prophylactic and curative activity are characteristics of this class of compounds — that is, derivatives of 6-methoxy-8-aminoquinolines — and not of only one member of the class. The demonstration of the latter feature of the antimalarial activity of the 8-aminoquinolines necessitated the use of these compounds at relatively high dosage. Incidental to these studies, another serious manifestation of 8-aminoquinoline intoxication was uncovered. Whereas pamaquine in high dosage (90 mg. of base per day), taken in conjunction with quinine, systematically produces a profound depression of white cells, it was found that other 8-aminoquinolines at a comparable dosage had the ability to produce a complete loss of the granulocytes — the cells that mobilize to fight acute infection. The occurrence of this in a group of patients receiving SN 8233 led to a revision of the manner in which 8-aminoquinolines were studied.

All work on the prophylactic action of the 8-aminoquinolines was stopped at that time, since it had been demonstrated for at least three derivatives (SN 971, SN 1452, and SN 11,191) that complete prophylaxis required daily dosage of drug in excess of that which could be given with safety. The systematic survey of 8-aminoquinolines for curative action was then organized in a fashion that permitted the screening of a relatively large number of compounds with safety and with a fair chance of selecting any very promising compound. It was assumed on the basis of available evidence that the highest daily dose of pamaquine that can be given with any safety in conjunction with quinine is approximately 90 mg. of pamaquine base; that since pamaquine produced moderate symptoms of intoxication at daily doses of 30 mg. of base, or one third the maximal tolerated dose, a generally useful 8-aminoquinoline would have to be effective at one sixth the max-

imal tolerated dose; that one could predict the maximal tolerated dose of an 8-aminoquinoline in man within a factor of two on a limited series of toxicity studies in the monkey; and that it was possible to screen out, in toxicity studies in the monkey, such compounds as possessed the irreversible type of damage to the central nervous system that characterized toxicity in certain members of the 8-aminoquinoline series.

On the basis of these assumptions, 8-aminoquinolines were selected for clinical trial if they were no more toxic than pamaquine. They were tested in combination with quinine in mosquito-induced Chesson *vivax* malaria at a daily dosage of one sixth their calculated maximal tolerated dose. Combined drug administration was continued for fourteen days in all cases. In the course of an exceedingly intense exploration of the 8-aminoquinolines, which has occupied a major portion of the entire program for the last year and a half, a great deal of valuable information was accumulated and a number of particularly useful curative agents were developed.

Curative tests in avian infections were devised in the hope of finding a close correlation between the response of avian and human parasites to pamaquine analogues. The degree of correlation was consistently disappointing, and in the end reliance was again placed on the action of a compound against the trophozoites in the bird as a nonspecific expression of biologic activity. Used in this broad way and with many reservations, these screening tests served as useful guides.

In order to provide adequate clinical facilities for the rapid curative and prophylactic tests of a number of compounds against *vivax* malaria, new projects were opened in three penal institutions and approximately five hundred additional volunteer subjects were used. In order to supply adequate quantities of infected mosquitoes for these enormous undertakings, new insectaries were established and mosquitoes were fed on specially selected subjects whose infections were particularly suitable for transmission of the disease to the mosquito.

Out of this enormous effort, several compounds have emerged that, at this writing, appear to cure the most virulent *vivax* infections with a minimum of toxic reactions, and a number of others with great promise are awaiting test.

The opportunities for further study in this series of compounds alone are still great, despite all the work that has gone before, and the result of these studies may bear fruit in aiding the eradication of other diseases by chemical means.

Part Nine: Penicillin

CHAPTER LII

PENICILLIN: A WARTIME ACHIEVEMENT

CHESTER S. KEEFER

IN THE SUMMER of 1941, when the Committee on Medical Research came into being, there was not enough penicillin in this country to treat a single patient. In the spring of 1942, a sufficient amount had been produced to treat *one* patient adequately. A year later the first group of wounded men returning from the Pacific received penicillin. By D-Day there was enough penicillin available for our armed forces and our allies, as well as a moderate amount for civilians. In April 1945, penicillin was removed from governmental allocation so that it could be used widely in this country and abroad.

The story of this achievement is one that has been told and recorded before, but it is proper that this volume should contain an account of the steps that were responsible for the development of penicillin from a laboratory curiosity to one of the most widely used anti-infective agents of all time.

Penicillin was discovered by Fleming in 1929, but it remained for Florey and his associates, Chain and Heatley, to concentrate and dry the active substance so that it could be given to man by injection without side effects. In 1940 the task of producing enough penicillin to treat a single patient was a stupendous one, and owing to the pressure of the war in England it was impossible for Florey and his associates to do anything about improving production promptly. In that year Florey and Heatley came to the United States with the hope of inducing American pharmaceutical houses to undertake the development of this vital material on a large scale.

Florey first interviewed Dr. R. G. Harrison of the National Research Council, who referred him to Dr. Charles Thom of the United States Department of Agriculture, who was the first to identify the mold that produced penicillin. Dr. Thom suggested that the group at the Northern Regional Research Laboratory of the Department of Agriculture, at Peoria, Illinois, was best qualified to give advice concerning penicillin production. The story of the work at this laboratory under the direction and leadership of Dr. Robert Coghill is told in the following chapter.

When Florey returned from his visit to Peoria, he consulted Dr. A. Newton Richards, Chairman of the Committee on Medical Research, and discussed the problem with him. At the beginning, Dr. Richards and the Committee realized that if penicillin fulfilled Florey's expectations, its development and production would be of immense value to the armed services. It was also appreciated that if enough penicillin was to be produced for thorough clinical testing, this could be accomplished only with the collaboration of the research and engineering facilities of commercial firms.

In the autumn of 1941, the Committee on Medical Research called meetings of the commercial companies known to be interested in penicillin production. These meetings placed on record the government's view that the matter was urgent and that commercial companies must be relied on to solve the problems of penicillin supplies.

The task seemed stupendous, since the yield of penicillin was so low and its production so difficult. The various commercial companies set to work to solve many of the problems, and the Office of Scientific Research and Development entered into contract with the Bradley Polytechnic Institute of Peoria, Illinois, so that additional resources might be available for necessary fundamental research by Dr. Robert Coghill and his associates at the Northern Regional Research Laboratory.

This research was necessary in order to ascertain what strains of penicillium were the most suitable for penicillin production, to discover the most favorable medium and conditions for growth, and to develop methods of extraction that would reduce the losses of active substance and at the same time purify the penicillin so that it could safely be given to man. All the information derived from the work of Coghill and his associates was promptly transmitted to commercial firms, and was added to the knowledge that was emerging from their own fundamental research. These research projects were fruitful. By careful selection of strains and improvement of the medium, the yield of penicillin in surface growth was increased one hundred fold. Later, by developing a new strain and studying conditions of its growth in submerged culture, these investigators laid the basis for the present large-scale production by culture in 1000- to 15,000-gallon tanks instead of in 1-liter bottles.

By January 1942, it had become apparent that small supplies of penicillin would be available for clinical investigation and trial within six months. The Committee on Medical Research requested the Committee on Chemotherapeutics and Other Agents of the National Research Council to arrange and supervise the testing and to collect information concerning the therapeutic value of penicillin. Owing to great difficulties in production, only enough penicillin to treat ten patients had been put out by June 1942. The Committee realized at the outset that in dealing with an extremely lim-

ited supply of such valuable material it would be necessary to restrict its use to the cases that would yield the maximum information of value to the armed services, and to those in which the drug was considered likely on the basis of preliminary tests to be of therapeutic value.

With these necessities in mind, a few experienced investigators were selected, on the basis of their knowledge and familiarity with the technics and skills involved in the clinical investigation of infections, to test penicillin in proved cases of the infections then under investigation. The reports of all cases were forwarded to a central office, so that all investigators, producers of penicillin, the Committee on Chemotherapeutics and Other Agents, and the Committee on Medical Research were familiar at all times with the results being obtained. These studies yielded fundamental information about absorption and excretion, the best methods of administration, and the necessary dosage, together with the frequency of administration, of penicillin. They also made it possible to assess its potential toxicity and to determine its field of effectiveness. It was absolutely essential that all the penicillin that was made available be used with the greatest of care and with thoughtful planning. In order to use this material with the greatest economy, it was necessary to set up priorities for its use.

From June 1942 until February 1943, enough material was made available to study only 100 patients. All the penicillin for this study was provided by three producers free of charge. Beginning in the latter month, the unfairness of this plan was apparent, so that from that time on the government paid for all the penicillin used in clinical investigation under the direction and supervision of the Committee on Chemotherapeutics and Other Agents. No individual doctor or patient could buy penicillin, and no patient was charged for it. Penicillin was issued to so-called accredited investigators with this understanding, and only on condition that they return complete reports to the Committee for analysis.

It was not until the following April that an opportunity to study the effect of penicillin in war wounds presented itself. This study was especially important and was carried forward by Dr. Champ Lyons at the Bushnell General Hospital in Brigham, Utah, with the full co-operation of the Army Medical Corps. An arrangement was made by the Chairman of the Committee on Medical Research with the Surgeon General of the Army for Dr. Lyons, then a civilian, to go to Utah. He found excellent collaboration from the commanding officer of the hospital and his entire staff, and the patients available were ideal for a thorough clinical test of penicillin in war wounds; that is to say, these patients were not responding well to the best treatment available at the time. The results of these studies were so striking that they could not fail to convince the medical corps of the armed forces and others that the drug possessed military importance of the highest order.

After this experience, Dr. Lyons was transferred to the Halloran General

Hospital in New York, where an extensive Army program of officers' train-ing in the use of penicillin began; this was continued in other Army hos-pitals in this country. Later Dr. Lyons was commissioned as a medical officer in the Army and was stationed in the Mediterranean Theater, where his work in the indoctrination of medical officers in the use of penicillin was outstanding.

In addition to programs of research for testing the efficacy of penicillin in the treatment of war wounds, other diseases of military significance were investigated under the Committee. The original observations of Dr. John Mahoney and his associates of the United States Public Health Service on gonorrhea and syphilis led to the adoption of penicillin as the drug of choice in the treatment of venereal disease in the armed forces. Studies were also carried forward in other diseases known to be resistant to the sulfona-mides.

Once it was established on the basis of study of a few cases that penicillin not only fulfilled the early expectations but exceeded them, the War Pro-duction Board consented in June 1943 to become responsible for the pro-gram of commercial production. Plans were immediately made for expand-ing production, and from July 16, 1943, until April 1945, the production and allocation of penicillin were the responsibility of the War Production Board. That this plan was successful everyone knows. Between June 1943 and June 1945 there was an increase of twenty-five hundred times the total production per month. The present production greatly exceeds the highest monthly rate achieved in that period. With this increase in production, the cost of penicillin was decreased from $200 to $6 per million units, and there was an adequate supply for the Army and Navy and our allies, as well as a supply for civilians.

When larger amounts of penicillin became available in August 1943, the War Production Board allocated to the Office of Scientific Research and Development increasing amounts of the drug as production increased, so that the program of clinical testing could be accelerated and the facts that would be of value to the armed services and civilians could be gathered as rapidly as possible. The organization for distributing penicillin for purposes of investigation and for collecting information was centralized in the office of the Chairman of the Committee on Chemotherapeutics and Other Agents and the Consultant to the Committee on Medical Research (Dr. Chester S. Keefer), at the Massachusetts Memorial Hospitals in Boston. All requests received from physicians and others were carefully considered. If penicillin was available and if the disease fell into one of the groups of diseases that were being studied at the time, the case was included in the investigation. Arrangements were made to forward the penicillin to the physician for the treatment of his patient, with the understanding that complete reports of the case would be made.

This program of clinical investigation was continued until April 30, 1944, by which time a sufficient amount of information had been accumulated on the clinical value of penicillin in certain diseases to justify a revision of the program. Also, by this time a sufficient amount of penicillin was being produced so that the War Production Board was able to meet military requirements and to allocate a small amount of penicillin to various hospitals throughout the country for use on patients who would be benefited by it.

At the beginning of the clinical trials, only five groups of investigators were selected to carry on the studies, and only three different infections were selected for investigation. With the increase in available supplies the number of accredited investigators working in various medical clinics and centers was increased to one hundred and fifty-nine, and the number of different groups of infections that were finally included in the investigations was sixty-seven.

Between August 1, 1943, and April 30, 1944, individual practicing physicians were included in the investigative program on the same basis as the workers who had been selected by the Committee for the study of specific problems. During this period requests were received from 9750 physicians, and penicillin was released to 4127 for the treatment of 5516 patients. The central office placed a total of 15,127 orders with various companies from February 1943 until April 30, 1944. Up to February 1943, 143 patients had been studied. The number of patients treated under the program of clinical investigation that expanded from that time was approximately 15,000. The results of this investigation were promptly made available to the armed services as well as to civilians.

The amount of penicillin allocated to the Office of Scientific Research and Development in the first seven months of 1943 was 443,500,000 units. In August 1943, 285,000,000 units were allocated for clinical investigation, and the total was gradually increased so that in April 1944, 3,310,000,000 units were allocated. The total amount allocated from August 1943 to April 1944 was 12,092,000,000 units.

The benefits that flowed from the study and development of penicillin as a chemotherapeutic agent may be summed up briefly as follows:

(1) It has reduced the death rate of staphylococcal infections with bacteremia from 75 to 10 per cent and has lowered the total number of days of illness in these infections immeasurably.

(2) It is the most effective agent available for the treatment of hemolytic streptococcus infections.

(3) It is as efficacious in cases of lobar pneumonia as the sulfonamides, and under certain conditions it is the agent of choice.

(4) It is the most powerful therapeutic agent available for the treatment of venereal diseases such as gonorrhea and syphilis, and has been adopted by the Army and Navy as the standard method of treatment of these diseases.

(5) In infected wounds and burns, penicillin when combined with good surgical treatment is an effective weapon in limiting infection and accelerating healing.

(6) When combined with antiserum and surgical débridement it is helpful in the treatment of gas gangrene.

(7) It is the best drug available for the treatment of such diverse diseases as pyogenic meningitis, bacterial endocarditis and mastoiditis, empyema, lung abscess and bronchiectasis, acute and chronic osteomyelitis, and anthrax.

There are still other diseases in which it shows great promise in experimental infections in animals, but opportunities for extensive clinical trial have not been available.

In essence, it can be said that penicillin is the most remarkable of all the chemotherapeutic agents. It is truly extraordinary that this substance should have such a powerful effect on so many different infectious agents and yet be nontoxic. When one reflects on the fact that it was only four years ago (at the time of writing) that the first patient was treated adequately in this country and that now there is enough material to meet all needs, it is a source of satisfaction to know that such a development can take place in our country during wartime. This achievement is the finest example of what can be accomplished by collaborative efforts in research and development in the medical sciences when the proper leadership and co-operation are available.

RESEARCH IN THE DEVELOPMENT OF PENICILLIN

KENNETH B. RAPER

R ESEARCH on penicillin at the Northern Regional Research Laboratory [1] in Peoria, Illinois, began on July 15, 1941, with the arrival at the laboratory of Professor H. W. Florey and Dr. N. G. Heatley of Oxford University, England. These investigators and their associates had been studying the production and evaluation of penicillin as a chemotherapeutic agent for more than two years. Their first paper, in which tests with laboratory animals were reported, had been published almost a year before. Their second paper, in which were reported the first and highly promising results in man, was already in press and was to appear a month later. Supported by a grant from the Rockefeller Foundation, they had come to the United States to find facilities to conduct pilot-plant scale experiments on the production of penicillin, since conditions in England at that time were unfavorable for the performance of such work.

Earlier in July, Florey and Heatley had applied to the National Research Council in Washington for assistance and had been referred to Dr. Charles Thom, in the Bureau of Plant Industry, United States Department of Agriculture. Thom, who was familiar with the facilities at the Northern Regional Research Laboratory, made arrangements with the Bureau of Agricultural and Industrial Chemistry to have the visitors go to the laboratory and consult with the staff members regarding their requirements. This conference was held on July 14, and with the assistance of Dr. R. D. Coghill, head of the Fermentation Division, the following plans were formulated.

It was decided that the Fermentation Division should pursue three lines of work, one or all of which were believed likely to lead to substantial increases in the production of penicillin. First, the possibility of producing the antibiotic by growing the mold submerged would be investigated. Second, by the use of surface-culture technics, attempts would be made to improve the yields of penicillin by studying the effect of changes in the culture medium, temperature, and other environmental conditions. Third, other molds

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

closely related to Fleming's isolate of *Penicillium notatum,* the only culture then definitely known to produce penicillin, would be investigated for their capacity to produce the bacteriostatic substance. Finally, it was agreed that Heatley should remain at the laboratory for two months to acquaint the staff with the production methods and culture technics used in England and, more particularly, to teach them the details of the bioassay that he had developed for testing the potency of culture solutions and concentrates.

It was realized that the first of these objectives was probably of the greatest potential importance as a means of increasing production. Nevertheless, it was felt that additional information regarding the nutrition of the mold in relation to penicillin production should first be obtained, and it was thought that this could best be accomplished in small surface cultures. The problem was assigned to Dr. A. J. Moyer, microbiologist in the Fermentation Division. In the succeeding months, assisted by Heatley, who performed the necessary assays, Moyer studied the effect on penicillin production of many different nutrients, including different salts and various sources of carbon and nitrogen. By September definite progress had been made, and arrangements were worked out with the Rockefeller Foundation to enable Heatley to remain at the Northern Laboratory for an additional three months.

The results of the work completed during this period were prepared by Moyer and Heatley for publication in *Science* early in December, but publication was withheld because of the developing war situation and the possible bearing that this information might have on national security. The report was submitted to the Committee on Medical Research for its information and for distribution to pharmaceutical firms then investigating the production of penicillin.

In the meantime, Dr. A. N. Richards, Chairman of the Committee on Medical Research, called a meeting of parties interested in penicillin production to be held in New York City on December 17, 1941. In attendance at this meeting were representatives from Merck and Company, Squibb and Company, Charles Pfizer and Company, Lederle Laboratories, the United States Department of Agriculture, and the National Research Council, with Dr. Richards as chairman. Dr. Coghill represented the Northern Laboratory and summarized the work on penicillin that had been done there.

Two important disclosures were made. First, of many nutrients known to favor the growth of micro-organisms that had been tested for their effect on penicillin production, concentrated corn steep liquor, a byproduct of the wet corn milling industry, had proved especially beneficial. By adding as much as 60 ml. of this material per liter to a modified Czapek nutrient solution, yields of penicillin up to 30 units per milliliter were obtained in the filtrate in five days at 24–25° C. This represented more than a ten-fold

increase over the yield that had been obtained in England by Florey and his associates. Second, preliminary experiments with submerged cultures had been made. Yields of penicillin up to 4 units per milliliter had been obtained in four days in Jena glass gas-washing bottles, using a steep liquor-enriched medium.

The report from the Northern Laboratory was enthusiastically received. Later, when Dr. Coghill indicated that with the departure of Dr. Heatley the work on penicillin might have to be curtailed, because of limitations in personnel and the necessity of concentrating on other projects then believed to be more important to the war effort, Dr. Richards offered to recommend the allotment of funds by the Office of Scientific Research and Development in whatever amount was deemed necessary to bring these investigations along at as rapid a rate as possible. As a result, approximately $8000 was appropriated. The application for this allotment outlined a plan of work representing in effect an extension of the investigations already in progress, and calling for the employment of two professional assistants and one laboratory helper to assist in the preparation of cultures and, more particularly, in performing the bioassays essential to the work.

Arrangements were made for the Bradley Polytechnic Institute in Peoria to provide for this service, working with the Fermentation Division of the Northern Laboratory. Under this contract, which became operative on February 1, 1942, the work was performed in the Fermentation Division, with Dr. Coghill as the responsible investigator. The contract covered a period of six months and was subject to renewal. In announcing it, Dr. Richards expressed the hope that the plan of work would include a standardization of the method of assay that could be used by producers of penicillin. This was subsequently done. The potential importance to the enemy of information obtained under this contract was recognized, and it was agreed that periodic reports would be submitted to the Committee on Medical Research to ensure safe and proper distribution.

Substantial progress was made during the ensuing six-month period. Further improvements in the culture solution were made, and yields of penicillin in surface culture were increased to 50–60 units per milliliter in five or six days.

All strains of *P. notatum* and closely allied species of molds contained in the Culture Collection of the Northern Laboratory were surveyed for their capacity to produce penicillin. Of forty-one strains thus examined, twenty-five showed little penicillin production and sixteen produced yields ranging from 8 to 16 units per milliliter. No strain was found to be better for surface production than the Fleming culture, but it was obvious that the capacity to produce penicillin was not a unique characteristic of this mold.

In the meantime, another strain of *P. notatum* (NRRL 832), which had not appeared very promising in surface cultures, gave excellent results when

grown submerged. On a modified Czapek's solution containing 2 per cent brown sugar and 3 per cent corn steep liquor, this strain produced 20–25 units per milliliter in shaken flask cultures in six days at 24–26° C., whereas the best substrains of the Fleming culture produced only 9 units under comparable conditions. It thus appeared that certain strains of Penicillium might be better adapted for surface production, while others might be better suited for submerged production.

Methods of assaying penicillin were greatly improved. Some attention was given to the serial-dilution method of assay, and improvements were made that substantially increased the accuracy of this method. More detailed study, however, was given to the cylinder-plate method, which had been developed by Heatley and was used in the studies of Florey and his co-workers. A number of factors were found to influence markedly the size of the circles of inhibition, and hence the correctness of the assays. So far as possible these variables were standardized and controlled, with resulting improvements in accuracy, and the procedures worked out were generally adopted in other laboratories.

The results obtained up to this time indicated that additional improvements in yield could be effected by continuing the investigation, and the possibility of producing penicillin on a commercial scale appeared extremely promising. The need for studies on the recovery, concentrations, and purification of the drug was becoming increasingly apparent. At the same time, and owing to the developing war situation, estimates of the amount of penicillin that might be required for military and civilian use were steadily increasing. There was no question, therefore, regarding continued support of the work by the Office of Scientific Research and Development. A supplementary contract became effective on July 31, 1942, to cover work during the ensuing year, and a second supplement was issued in July 1943, to continue support until July 31, 1944.

During this two-year period, the Department of Agriculture was allotting funds for the investigations and assigning personnel to it on an expanding scale, and this was continued until November 1, 1945, when the investigation was terminated. Altogether, something over $27,000 was expended by the Office of Scientific Research and Development on the penicillin investigations at the Northern Laboratory through its contacts with Bradley Polytechnic Institute between February 1, 1942, and July 31, 1944. In the same period and during the fifteen months following, the Department of Agriculture was estimated to have spent $100,000 on the project.

Even after the financial support of the Committee on Medical Research was no longer needed, all progress reports continued to be submitted to it for distribution. The work was thus jointly sponsored and supported, and from beginning to end it was handled as a single investigation. The more

outstanding achievements resulting from this common endeavor are described below.

From January 1943 to November 1, 1945, Coghill acted as consultant to the Office of Scientific Research and Development, assisting the Committee on Medical Research in its task of co-ordinating penicillin research in this country and in advising the Drug and Cosmetics Division of the War Production Board regarding the number and types of installations necessary to produce the amount of penicillin required to meet estimated military and civilian needs. In his dual capacity as head of the Fermentation Division of this laboratory and as consultant, Coghill contributed greatly to the whole penicillin development in this country.

DEVELOPMENT OF THE CORN STEEP–LACTOSE PRODUCTION MEDIUM

Studies on the development of an optimum culture medium for penicillin production were continued by Moyer. Further investigations confirmed the beneficial effect of corn steep liquor,[2] and at levels below those of actual toxicity (usually 9–12 per cent by volume) yields of penicillin in surface cultures were found to increase with the addition of increased amounts of this material. In attempts to find other nitrogenous nutrients productive of high yields of penicillin, various substances were investigated, including animal and vegetable extracts, soybean meal, amino acids, singly and in mixtures, and protein hydrolysates. Better yields were obtained with trypsin-

---

[2] In the preparation of corn for starch production, it is first steeped for thirty-six to forty-eight hours in a 0.1–0.2 per cent aqueous solution of sulfur dioxide at 52–54° C. The water employed in this process is generally that previously used on the starch washing tables and in the gluten settling tanks, and therefore already contains considerable dissolved material. During the steeping process, additional solubles from the fresh corn are leached out and a mild lactic acid fermentation takes place. The steep water therefore contains corn solubles from all stages of the manufacturing process, together with products resulting from the lactic fermentation. The steep water as it is drained from the corn is concentrated to a thick syrup, containing 50–55 per cent dissolved solids, and is marketed as corn steep liquor or added to the stock feed, which is an important byproduct of the corn milling industry. Different commercial lots of corn steep liquor vary substantially in composition. The analyses of the three samples listed below may be regarded as typical of such variation.

	Sample A	Sample B	Sample C
pH	4.0	3.95	4.1
Specific gravity	1.250	1.255	1.260
Total N (gm./100 gm. steep liquor)	4.3 gm.	3.74 gm.	4.06 gm.
Amino-N (gm./100 gm. steep liquor)	——	0.685 gm.	1.10 gm.
Ammonia-N (gm./100 gm. steep liquor)	——	0.0065 gm.	0.019 gm.
Reducing sugar (as glucose)	5.64 gm.	5.62 gm.	2.27 gm.
Ash	7.86 gm.	9.11 gm.	10.68 gm.
Lactic acid	——	10.8 gm.	16.7 gm.
Total solids	52.0%	51.1%	45.0%

hydrolyzed casein than with any other substitute, but even here the yields were only half those obtained with corn steep liquor under comparable conditions. Repeated attempts to fractionate the steep liquor and thereby develop a simpler and more reproducible medium were likewise unsuccessful. In all tests, highest yields were obtained when the whole steep liquor was used, and we were led to believe that its effect was multiple in character, involving mold nutrition, pH regulation, and, as later appeared probable, the supplying of an actual building block (phenylacetic acid) for the synthesis of the penicillin molecule. Of various inorganic nitrogen sources investigated, sodium nitrate used in a concentration of 0.3 per cent proved most satisfactory. The effect of trace elements was studied, but none was found to increase yields substantially.

Next to steep liquor, the most important ingredient in the medium was the carbon source. As a substrate for penicillin production, glucose had the disadvantage that it was rapidly metabolized with the production of acid by *P. notatum*. This made it difficult to keep the reaction within the range pH 6.5–7.5, where it was known that penicillin production was at its maximum. A study was therefore made of carbon sources that would presumably not yield an acid on oxidation by *P. notatum*.

Under the conditions used, mannitol, sorbitol, and lactose appeared to be the best sources of carbon. As better organisms were later developed and as the functions of various constituents of the medium were better understood, it became apparent that lactose was the best and most available of these three. The lactose, as indicated by the amount of fungus growth and the pH of the medium, was not so readily attacked as glucose or the other carbon sources tested. It was also noted that the penicillin titer did not fall off as rapidly in media containing lactose as in the other media. Tests were undertaken to ascertain how pure the lactose had to be for purposes of good penicillin production. Many samples from different lactose producers were given test runs, and it soon became obvious that even the poorest grades of lactose, including a sample analyzing as low as 88 per cent (too low to be sold as lactose), gave satisfactory yields of penicillin. This made it possible to manufacture a lactose for the penicillin producers without having to install expensive equipment for the several recrystallizations usually necessary.

Since it appeared that the manufacturing capacity of the country's lactose producers might not be sufficient to supply the demands of the developing penicillin industry, other carbon sources that were slowly utilizable by the mold were investigated. It was found that for the production of penicillin in surface cultures, starch, modified starch, ground grain, or grain worts gave satisfactory yields. These materials, however, were somewhat more difficult to handle, and as things worked out enough lactose to supply the penicillin producers soon became available.

While no single quantitative formula can be cited as the optimum medium for surface production of penicillin, a medium of the following composition may be regarded as approximating such a standard.

Corn steep liquor (concentrated)	80.0 ml.
Lactose	40.0 gm.
$MgSO_4.7 H_2O$	0.25 gm.
$NaNO_3$	3.00 gm.
KH_2PO_4	0.50 gm.
Zn (as sulfate)	0.01 gm.
Water to make	1.0 l.

On media of this general composition, and utilizing improved strains such as NRRL 1249.B21, to be discussed later, yields of penicillin up to 180–200 u./ml. were obtained in six to seven days in laboratory experiments, using small Erlenmeyer flasks as culture vessels. In large installations yields were consistently lower but commonly exceeded 100 u./ml. in the harvested broth. With minor variations in the amounts of ingredients used, depending on individual preferences and local conditions and equipment, and with the occasional substitution of starch for lactose, the above formula was generally employed in industry for the production of penicillin by the surface-culture technic as long as this method proved practical.

DEVELOPMENT OF SUBMERGED FERMENTATION

The surface-culture technic represented the simplest and quickest means of producing penicillin, especially for those without previous experience in the fermentation field. It required less critical materials, engineering problems were comparatively simple, and contamination, when it occurred, was generally not too serious, since it was confined to a limited number of small culture vessels. This method was of the greatest importance in the early days of the penicillin development. It was in this type of laboratory culture that basic facts regarding the nutrition of the mold in relation to penicillin production were worked out, and it was by this method of manufacture that almost all the penicillin used to establish its clinical usefulness was produced. Nevertheless, it was realized from the outset that if penicillin could be produced in submerged culture, as is done with gluconic acid from *Aspergillus niger,* this would represent the best means of achieving large-scale commercial production at a reasonable price. Assuming equal yields by the two methods, a single 10,000-gallon tank would represent the equivalent of 60,000 to 70,000 two-quart bottles in production capacity, and would involve infinitely less manual labor and obviate the necessity of installing large and expensive incubator rooms.

It was early determined by Moyeo and Coghill that penicillin could be produced by *P. notatum* when grown submerged, and that certain strains,

notably NRRL 832, appeared to be especially suited for this type of production. Yields, however, were disappointingly low when compared with those then obtained in surface cultures. To overcome this differential, the nutrient requirements of the mold when grown submerged were carefully examined. It was found that a lactose-corn steep liquor medium could be used most satisfactorily, but that the concentration of these two principal ingredients needed to be about half that employed for surface cultures, and yields of penicillin were consistently improved by the addition of about 1 per cent sterile CaCO₃ to the cultures at the time of inoculation. On the basis of laboratory experiments in shaken flask cultures, a medium of the following composition was recommended as one of the best for producing penicillin in submerged culture:

Corn steep liquor	40.0 ml.
Lactose	27.5 gm.
NaNO₃	3.0 gm.
MgSO₄.7 H₂O	0.25 gm.
KH₂PO₄	0.50 gm.
ZnSO₄.7 H₂O	0.044 gm.
MnSO₄.4 H₂O	0.020 gm.
Glucose	3.0 gm.
Water to make	1.0 l.

By employing this medium and incubating the cultures at 24–25° C., yields of penicillin up to 75–80 u./ml. could be obtained in six days in small flasks, which were continually shaken to provide agitation and necessary aeration.

Whereas nutritional studies could be satisfactorily made in small-flask cultures, it was obvious that more critical evaluation of the submerged method would depend on tests in larger-scale equipment. Successful runs of this type made at the Northern Laboratory in rotary drum fermenters were reported in monthly Progress Report No. 3 to the Committee on Medical Research. Yields of 7–9 u./ml. were obtained. Thereafter, tests in this equipment and in larger vat fermenters were run from time to time as new developments occurred in the smaller-scale experiments referred to above. Later, when new and more productive strains were being isolated and developed, this type of investigation was greatly intensified; it had become increasingly evident that more knowledge was needed regarding environmental factors such as aeration and agitation, as well as about important chemical changes taking place in the culture medium during the course of the fermentation. This phase of our investigation was carried out in the Fermentation Division, and the results were made available to the producers through the periodic reports to the Committee on Medical Research. Briefly summarized, it was eventually found that with the more productive strains

such as *P. chrysogenum* (NRRL 1951.B25 and NRRL 1984.A) yields of penicillin up to 250–260 u./ml. could be obtained in three or four days in a medium containing 2.5–3.0 per cent lactose, 6 per cent concentrated corn steep liquor, and 0.25–0.5 per cent $CaCO_3$. The use of mineral salts was found to be optional. The solutions were vigorously agitated, and sterile air was supplied at a rate of ½ to 1 l. of air per liter of culture solution per minute. Excessive foam was held in check by an antifoam agent, usually lard oil containing about 1 per cent octadecanol. All fermentations were run under positive pressures. Employing strain X–1612, an x-ray-induced mutation of NRRL 1951.B25, characterized by increased productive capacity, yields up to 350 u./ml. were subsequently obtained under essentially the same operating conditions. The detailed results of this work have not been published, but are corroborated by the findings of the University of Wisconsin group.

INCREASED PENICILLIN YIELDS WITH PHENYLACETIC ACID

When it became apparent that phenylacetic acid was a constituent part of penicillin G, it was naturally postulated that an increased yield of penicillin might be produced by growing the molds in a culture solution to which this acid had been added. Initial experiments in surface culture indicated that phenylacetic acid in a concentration of about 0.05 gm./l. was toxic when added to a culture solution at pH 4.0, the initial reaction of the usual media prior to inoculation with the mold. If, however, the mold was permitted to establish a mycelium before phenylacetic acid was added, or if the initial hydrogen-ion concentration was adjusted upward to pH 4.6 or above, concentrations of phenylacetic acid up to 0.5 gm./l. were well tolerated and final yields of penicillin were substantially increased. The basic nutrient solution employed was essentially that listed on page 729, and the pH was adjusted by addition of KOH or NaOH. Phenylacetic acid of technical grade was as effective as material of higher purity.

The beneficial effect of phenylacetic acid in surface cultures is clearly evident from the results shown in Table I. Here, the addition of phenylacetic

TABLE I

Effect of Phenylacetic Acid in Surface Cultures

	Assay Organism	4th day u./ml.	5th day u./ml.	6th day u./ml.	7th day u./ml.
Control	*Staph. aureus* (B–313) ...	98	146	189	194
	B. subtilis (B–558)	—	160	192	189
+ Phenylacetic	*Staph. aureus* (B–313) ...	132	233	263	313
acid (0.4 gm./l.)	*B. subtilis* (B–558)	—	222	250	316

acid resulted in a 66 per cent increase in the penicillin yield at seven days. In this experiment *P. notatum* (NRRL 1249.B21), with or without the addition of phenylacetic acid, appeared to produce penicillin G under the culture conditions prevailing.

The net result of the development of improved organisms, such as NRRL 1249.B21, a better understanding of the functions of the various medium constituents, and the use of phenylacetic acid has been to increase the yield of penicillin in surface cultures from the 2–4 u./ml. obtained by the English workers, or the 20–40 u./ml. yields obtained in this laboratory at the beginning of the first contract, to over 300 u./ml.

The addition of phenylacetic acid to submerged cultures resulted in limited increases in yield. These, however, were less substantial than those obtained in surface cultures, and the addition of this adjuvant as a means of increasing yields under these conditions appeared of questionable significance. It is unfortunate that the discovery of the effect of phenylacetic acid was not made earlier, when most of the penicillin produced was being manufactured by the surface-culture method.

Months later, when the high-yielding mutant strains, X–1612 and Wis. Q–176, developed from *P. chrysogenum* NRRL 1951.B25 (see page 738), were generally adopted by industry for penicillin production and were found to produce primarily penicillin K, the addition of phenylacetic acid or phenylacetamide to the culture solution was found to favor the production of the more stable, and clinically more useful, penicillin G. The addition of these or other adjuvants to effect the same goal is now an accepted practice in industry.

REFINEMENT OF ASSAY METHODS

Parallel with investigations on the production, concentration, and purification of penicillin, efforts were continually made to improve the accuracy of the bioassay used to determine the potency, or titer, of the product.

Three principal methods have been suggested for the bioassay of penicillin: the cylinder-plate method of Heatley, the broth-dilution method, and the turbidimetric method. Although a limited amount of study has been devoted to the second of these methods, work at this laboratory has been concerned almost entirely with the first, which is not only the most widely used but, with modifications, is the method that is being currently used by the Food and Drug Administration for certifying commercial samples of penicillin.

The cylinder-plate method involves the use of a nutrient agar layer, about 3 mm. deep, the surface of which is heavily seeded with *Staphylococcus aureus*. Sterile cylinders are then arranged on the agar surface and filled with the solution of penicillin to be assayed. The plates thus prepared are

incubated at 37° C. until well-defined circles of inhibition are visible around the cylinders. The speed of penicillin diffusion outward from the cylinder is a function of the concentration of the penicillin contained therein, and the size of the resulting circles of inhibition can be used to prepare a standard curve by employing known strengths of a standard penicillin solution. The amount of penicillin in unknown samples can then be calculated by measuring their circles of inhibition and interpolating on the standard curve. At least two modifications of the method can be used successfully: cups may be cut in the agar to hold the penicillin solutions, and when carefully prepared appear to be as satisfactory as the cylinders; or filter-paper disks of standard diameter, thickness, and porosity may be dipped into samples and placed on the seeded test plate in lieu of filled cylinders. The latter modification is of more recent origin, is in many ways more convenient, and is currently finding wide acceptance in control laboratories.

As first taught to our workers by Heatley, many aspects of the cylinder-plate method were not adequately controlled. Some of these variable factors and the manner in which they have been largely standardized after more than three years of study are discussed below.

It was found that the composition and pH of the nutrient medium used in the agar plates must be accurately controlled, since these factors influenced the growth of the test bacterial species and the rate of diffusion of the penicillin. For example, larger circles were obtained in plates supporting a medium bacterial growth than in similar plates characterized by heavy growth. Also, much larger circles of inhibition were obtained in plates at pH 6.0–6.5 than at pH 7.0–7.5.

It was found that the depth of the agar in the assay plate influenced the size of the zones of inhibition, larger circles being obtained in plates, or areas of plates, characterized by shallower agar layers. This was undoubtedly due to the resulting higher concentration of penicillin in the smaller volume of agar within a given distance of the cylinder. To eliminate this variable, a standard measured amount of agar was added to each plate, and only plates with flat bottoms were employed. Subsequently, rectangular trays with plane (plate) glass bottoms were devised that effectively eliminated this variable. The use of such trays was first suggested and subsequently published by Beadle and his co-workers, and with some modifications was adopted in our laboratory.

It was found that the time lapse between filling the cylinders and placing the plates in the 37° C. incubator influenced the size of the zones of inhibition. For example, the diameters of these circles were increased 2 to 4 mm. by storing the plates for three hours in the refrigerator before transferring them to the incubator. This was probably because growth of the staphylococcus was inhibited by the low temperature while the diffusion of the penicillin was getting a head start. Smaller but significant errors resulted when seeded

plates remained at room temperature for appreciable lengths of time before the cylinders were filled. In the latter case, the bacteria made a limited growth before diffusion of the penicillin began, with a consequent reduction in the diameter of the zones of inhibition. To eliminate these variables a definite time schedule was established for seeding plates, filling cylinders, and initiating incubation at 37° C.

The strain of test organism used was found to be very important, since some strains gave more sharply defined zones than others. *Staph. aureus* (NRRL B–313; Food and Drug Administration strain No. 209) was found to give very sharp boundaries and was early adopted as the standard test organism in this and other laboratories. The strain is fairly stable in culture, and if proper precaution is observed in maintaining it, few difficulties traceable to variation are encountered.

The amount of inoculum used on the test plates was found to influence the size of the zones. This is related to the timing factor already discussed, since it markedly affected the rate of growth of the test bacteria. It is sufficient to say that the best results were obtained by using the minimum inoculum that would give confluent growth of the staphylococcus on the surface of the test plate.

When all possible precautions were observed, circles of inhibition still varied somewhat on different plates. It became standard procedure, therefore, to fill two or more cylinders on each plate with a standard of known penicillin concentration. Two of five cylinders constituted such standards when petri dishes were employed, and after the larger rectangular plates were adopted eight of twenty-four cylinders alternately placed on opposite sides of the plate served the same purpose. Assay values for the unknown sample or samples on any given plate were then calculated in terms of the standards of the same plate by raising or lowering the standard curve on its Y axis to pass through the point determined by averaging the standards for that plate. The standard penicillin solution employed was prepared each week from a crystalline preparation.

The detailed technics developed and employed to control and standardize the above variables have been published elsewhere and need not be considered here. It should be noted, however, that the Northern Laboratory did achieve a degree of accuracy in its assays that was seldom equaled and rarely surpassed in any other laboratory investigating penicillin.

As it became known that there were a number of different natural penicillins, and that two or more of these were sometimes produced in the same culture broth, considerable attention was given to the development of a differential assay for these penicillins. While nothing approaching a quantitative differentiation was ever achieved, a method was developed that when properly employed gave an indication of such mixtures. This was used with some success in determining the amount of chloroform-insoluble penicillin X,

which was produced by selected mold cultures, and aided greatly in the development of a high penicillin X-producing strain.

The application of this method hinged on differences in the inhibition of different species and strains of penicillin-sensitive bacteria. Table II illustrates the degree of difference in inhibition resulting when four crystalline penicillins are tested against two bacterial strains that have been much used in assays of this type.

TABLE II

Biologic Activities of Four Natural Penicillins against *Staph. aureus* (NRRL B–313) and *B. subtilis* (R) (NRRL B–558)

Penicillin Type	Activity in u./mg.		Ratio of Activity
	Staph. aureus	*B. subtilis* (R)	*B. subtilis* (R)/ *Staph. aureus*
G (II)*	1,667†	1,667†	1.0†
F (I)*	1,450–1,475	970±	0.65
X (III)*	900–950	1,200–1,900‡	1.4–2.0‡
K (IV)*	2,200–2,300	750±	0.33

* Designation employed in Great Britain.
† By definition.
‡ Depending on the phase of *B. subtilis*, with ratio increasing with the degree of roughness of the strain.

The amount of routine work necessary to perform the large number of assays demanded by our investigations was always great. The magnitude of this, however, was greatly reduced by the development of a number of automatic devices, including a cylinder-loading device, an assembly for making rapid pH determinations, automatic agar dispensers, and devices for increasing the speed and accuracy of measuring the diameters of inhibition zones. These mechanical aids, which also helped to standardize our methods and thus improved the accuracy of results, were developed primarily by Max D. Reeves.

THE DEVELOPMENT OF IMPROVED PENICILLIN-PRODUCING MOLDS

Among many strains tested during our early investigations, almost all were found to produce some penicillin, and although no other strain was found to equal the Fleming strain when grown in surface culture, a second strain of *P. notatum* (NRRL 832) was found to produce greater yields than the Fleming strain when grown submerged. It was thus apparent that the ca-

pacity to produce penicillin represented a group rather than a strain or specific character, and that different members of the group varied greatly in their capacity to produce this antibiotic.

It was likewise found that good penicillin-producing strains were often subject to considerable variation in laboratory culture, and that substrains of differing productivity could be separated out and maintained in culture. Strain NRRL 1249.B21, a derivative of the Fleming culture capable of producing about twice as much penicillin as the original isolate, was of monospore origin and was developed in this manner in 1942. This strain was noteworthy for its capacity to produce substantially increased yields of penicillin when grown in surface culture. It found immediate acceptance in this country and Great Britain, and has been employed wherever penicillin has been produced commercially by this method. It was likewise employed for the production of penicillin by the "bran process" and for the preparation of surgical dressings, a development that attained some proportions in Hawaii and the South Pacific area during the war.

The search for better penicillin-producing molds was rapidly expanded in 1943. At this laboratory a program was undertaken to isolate from nature as many representatives of the *P. notatum-chrysogenum* group as possible and to test these for their capacity to produce penicillin. Strains were isolated from food products, fruits, and vegetables, and from soils collected from numerous stations in the United States and in many foreign countries.

In the handling of these cultures, a simple screening test was developed that effectively weeded out the less productive strains, while the more promising were surveyed thoroughly in surface and in shaken-flask culture. A general correlation was observed between cultural and morphologic characteristics and penicillin-producing capacity of these newly isolated cultures. While no strains were isolated that exceeded NRRL 1249.B21 for the production of penicillin in surface culture, a limited number of strains were found that produced yields in excess of NRRL 832 when grown submerged. Of these latter cultures *P. chrysogenum* (NRRL 1951, isolated from a cantaloupe collected in Peoria, Illinois) was found to be extremely variable in culture, and from it a series of superior producing substrains was obtained, including NRRL 1951.B25 and the now justly famous strains X-1612 and Wis. Q-176. The capacity to produce penicillin in surface and in submerged culture was tested in lactose-steep liquor media of the approximate formulas listed on pages 729 and 730.

An intensive study of natural variation in selected strains was likewise undertaken as a possible means of securing even more productive types. Substrains of NRRL 1249.B21 were repeatedly isolated and tested, but none of these were found to be superior to the parent culture, and yields of penicillin declined markedly as the subcultures differed increasingly in any direction from the characteristic 1249.B21 type.

Attempts to isolate various substrains from NRRL 832 failed to produce any markedly superior penicillin-producing cultures, but did reveal some striking cultural variants, including one substrain characterized by a red to light purple coloration.

Outstanding success was realized in the development of high-yielding strains from NRRL 1951. When first tested, this strain produced yields in submerged culture slightly in excess of NRRL 832. Its potentialities were suggested when colonies in streak-plate cultures seeded from week-old shaken flasks developed conspicuous sectors, and they became evident when the subcultures from these were found to produce greatly increased yields, both in surface and in submerged cultures. Repeated cultivation and reisolation of new substrains from dilution plates and from sector variants in giant colonies yielded numerous high-producing strains. Of these, NRRL 1951.B25 appeared to possess a slight advantage, although it was neither culturally very different from some other isolates nor a markedly better penicillin producer. This strain produced yields in surface culture equal to or slightly in excess of NRRL 1249.B21 and was less sensitive to temperatures above 24–25° C., but its greater pigment production precluded its adoption by the surface-culture industry. Its principal value stemmed from its behavior in submerged culture, where yields approximately double those for NRRL 832 were usually obtained. Continued investigation of natural variations in strain NRRL 1951.B25, however, failed to reveal any subculture capable of producing higher penicillin yields than the parent stock, although the widest variety of cultural types were separated out and tested.

Through a similar selection of natural variants, higher-yielding substrains were subsequently developed from other basic stocks such as a culture of *P. chrysogenum* received from the University of Minnesota as No. R–13 and entered in our collection as NRRL 1984. In these cases, also, certain increased levels of productivity were reached beyond which no further improvement was made, although the selection and examination of naturally occurring cultural variants were continued.

Early in 1944, faced with the demand for ever-increasing amounts of penicillin, and fully cognizant of the progress that had been made in the development of more productive cultures at this laboratory, the Office of Production Research and Development of the War Production Board set up projects at various institutions to explore vigorously each of the several approaches to the problem of obtaining additional cultures characterized by increased penicillin production. A conference of representatives from each of these institutions was held at the Northern Regional Research Laboratory on April 28–29, 1944, to draw up a co-ordinated program of work. In this work, attention was directed primarily toward the development of better submerged cultures, since it was then obvious that this type of production was most feasible.

At the University of Minnesota attention was directed primarily toward the isolation of new strains from soil and other natural sources. A culture of *P. chrysogenum* of outstanding merit, Minn. R–13, was discovered, which was widely studied and from which industrially important substrains were developed. At the University of Wisconsin strain variation was investigated by one group and the metabolism of penicillin-producing molds by another.

At Stanford University and at the Carnegie Institution, attention was directed particularly toward the production of artificially induced mutations from known good strains, such as NRRL 1951.B25, by exposing conidia to ultraviolet and x-ray radiation. At Stanford alone, more than 60,000 isolates were tested and a limited number of strains were found to produce somewhat better yields than the parent stocks.

At the Carnegie Institution a much smaller number of cultures were irradiated, but of these one strain (X–1612) was produced that for a time, at least, warranted the title "super strain." The production of this culture represented a joint endeavor. The stock was supplied by the Northern Regional Research Laboratory; the irradiation was performed at the Carnegie Institution; the initial and indicative production tests were made at the University of Minnesota; and the real magnitude of the superiority of this strain was demonstrated at the University of Wisconsin in 80-gallon fermenters. Maximum yields obtained with the parent NRRL 1951.B25 ranged around 250 u./ml., while yields up to 500–600 u./ml. were obtained with the mutant, X–1612.

More recently, a much higher-yielding strain has been developed from X–1612 at the University of Wisconsin. By irradiating conidia of this mutant with ultraviolet, a mutant (Wis. Q–176) has been obtained that is capable of producing yields of penicillin in excess of 900 u./ml. under favorable conditions. Culturally and morphologically, this strain bears a striking resemblance to the parent strain X–1612 and the grandparent strain NRRL 1951.B25. The strain represents primarily a biochemical rather than a cultural or morphologic mutation. Like the parent strains, Q–176 is culturally unstable and tends to produce variants differing in cultural aspect and in penicillin production, but if adequate precautions are observed a highly productive stock can be maintained without serious difficulty.

When first used in industry, strains X–1612 and Wis. Q–176 were found to produce primarily penicillin K, a type that is inferior for therapeutic use. However, if phenylacetic acid, phenylacetamide, or some other suitable supplement to the basic lactose-steep liquor medium is added in appropriate amounts, the penicillin produced is primarily penicillin G, with total yields remaining at a high and satisfactory level.

The importance of the foregoing developments to the present high levels of penicillin production cannot be overemphasized, since current high yields of 750–900 u./ml. are obtained in nutrient solutions of approximately the

same composition as those used to produce maximum yields of 75–100 u./ml. with NRRL 832 three years ago.

After the possible therapeutic significance and the interesting chemical possibilities of penicillin X became known, we undertook to secure, if possible, a strain capable of producing substantial yields of this penicillin in submerged culture. While penicillin X was first isolated from surface fermentations with NRRL 1249.B21, and while the first material available for clinical use and chemical investigations came from this source, it was realized that a good submerged strain would be required if penicillin X was to be made in quantity. Several of our best-producing strains were investigated. Of these, NRRL 1984.A, a naturally occurring variant of Minn. R–13 selected at the Northern Laboratory, showed the highest ratio of penicillin X, amounting to more than 15–20 per cent by assay. Attempts to develop natural variants characterized by increased penicillin X production were unsuccessful. By irradiating conidia of the same strain with ultraviolet, however, a substrain, designated NRRL 1984.N22, was obtained that gave satisfactory total yields, of which approximately 50 per cent represented penicillin X. Total yields of approximately 200 u./ml. have been obtained in ninety hours in a 600-l. vat fermentation. As indicated by differential assay and as subsequently shown by actual isolation, penicillin X represented approximately 50 per cent of the total potency as measured in staphylococcus units per milliliter, or about 65–70 per cent of the total on a weight basis, assuming the balance to be penicillin G. While penicillin X is not known to be produced and marketed as such at the present time, the development of strain NRRL 1984.N22 should provide a means of producing this penicillin in quantity should the demand for a drug possessing its specific properties develop.

THE NORTHERN LABORATORY CULTURE COLLECTION

The presence of a large mold collection at the Northern Regional Research Laboratory was an important factor in first influencing Florey and Heatley to bring their problem to Peoria. In the period since that time, the Culture Collection of the Fermentation Division has rendered an outstanding service to penicillin producers and to research laboratories investigating this and other antibiotics, not only in developing new and more productive strains but in serving as a source of proved and authentic cultures. In July 1941, the collection contained about forty representatives of *P. notatum* and closely allied species. While this number may seem surprisingly small when viewed in terms of collections subsequently assembled, it nevertheless constituted the largest number of identified strains present in any collection at that time. By surveying this small reservoir of available types, two important facts were established: that the capacity to produce penicillin in varying amounts was

a characteristic of the *P. notatum-chrysogenum* group; and that certain strains were capable of successful adaptation to a submerged process of manufacture. Starting from these basic facts, the search for new and more productive types was subsequently undertaken, with the exceedingly successful results already noted.

During this same five-year period, more than one thousand cultures of penicillin-producing molds have been sent out from this collection. These have gone to the research laboratories of pharmaceutical firms producing penicillin, to university laboratories investigating the production of this and other antibiotics, and to official agencies of many allied nations charged with the responsibility of introducing or increasing the production of penicillin in their own countries. Among the nations to which such cultures have been supplied may be listed the following: England, Canada, Australia, China, Brazil, Colombia, Chile, Greece, Ecuador, India, Russia, Egypt, Palestine, Mexico, and, after occupation by the allied forces, Holland, Denmark, Italy, Belgium, France, and Hungary. While the number of cultures distributed provides a clue to the importance of this service, its real value must be measured in terms of the authenticity and performance of the cultures sent out.

As new and better cultures were developed, these were announced in our periodic reports to the Committee on Medical Research, and the cultures were supplied to all qualified investigators and laboratories as requests were received. The importance attached to this service may be gauged by the fact that we complied with more than fifty such requests for the single strain NRRL 1951.B25 during the eight-month period after it was first reported.

Cultural variation is characteristic of many of the outstanding penicillin-producing molds, and this basic instability has been a matter of great concern to investigators and plant operators alike. High-yielding strains often tend to produce sectors, or overgrowths, differing from the parent in rate and type of growth and usually in their capacity to produce penicillin as well. Such variation may be in the direction of increased yields, as in the origin of NRRL 1951.B25 from NRRL 1951 (see page 737), but much more frequently it is in the opposite direction. Once a high-yielding culture is developed, it is therefore the mark of discretion to maintain it in as nearly unaltered form as possible. The stock culture should be transferred rather infrequently, and in doing so a mass inoculum of many conidia should be used so that the resulting culture will represent a proper mixture of whatever variant types may be resident in it.

In our work, three methods were found to be signally useful in minimizing strain variation: preservation of conidia in lyophil form, preservation of conidia in sterilized soil, and preservation of multiple agar slant cultures seeded from a uniform spore suspension. The first of these methods was especially recommended because of the greatly extended period of spore

viability, because new growth can arise only from the spores initially processed, and because there is no possible danger of contamination during storage. The repeated requests received from particular sources for new cultures of the same strains at successive intervals may be regarded as indicating the success achieved with these methods.

INVESTIGATIONS OF PENICILLIN STABILITY

The instability of penicillin was reported by Fleming in his original paper announcing its discovery in 1929. It was likewise reported to be extremely labile by Raistrick and his co-workers in 1932, and its instability at raised temperature was an important factor limiting the scope of their investigation. However, aside from the general recognition that elevated temperatures and excess acidity or alkalinity caused rapid inactivation of the bacteriostatic principle, little work was reported covering inactivation at different temperatures or over wide ranges in pH. The importance of such information to the development of successful recovery processes soon became evident. To supply such data a detailed and comprehensive study of the effects of temperature and pH was undertaken.

Two comparatively pure penicillins were studied in this work: a crystalline sodium salt obtained by the submerged growth of NRRL 832, and a partially purified sodium salt derived from surface growth of NRRL 1249.B21. The so-called "832 penicillin," subsequently identified as primarily penicillin G, assayed 1450–1500 u./mg., and the "1249 penicillin," subsequently identified as primarily penicillin F, assayed 900 u./mg. In addition, a partially purified solution of 832 penicillin, assaying 2200–2400 u./ml., was also checked for stability.

Carefully weighed samples of penicillin were dissolved in buffer solutions at the desired pH and temperature. After obtaining zero time samples for dilution, subsequent samples were taken at regular intervals and instantly raised to pH 6.0 with previously chilled K_2HPO_4–KH_2PO_4 buffer. Sampling periods ranged from three-minute intervals at pH 2.0 and 30° C. to daily samples at pH 5.0 and 10° C.

When penicillin concentrations were plotted against time on logarithmic paper, the points all fell on a straight line within experimental error. The figures, which were subsequently published, adequately portrayed the known instability of penicillin. For instance, at pH 2.0 and 24° C. the half-life of penicillin G is seventeen minutes. At 0° C. it is somewhat more stable, but it is half destroyed in approximately five hours. However, if the pH is raised to 3.0, the half-life of the penicillin at 0° C. is thereby increased from five to twenty-six hours. At higher pH levels the stability of the penicillin is proportionately greater, with a maximum at approximately pH 6.0.

The returns on this tedious piece of work resulted in increased yields of

penicillin following proper modifications of plant recovery processes. It was obvious that the pH must not be lowered any more than necessary, consistent with the removal of penicillin by the amount and distribution coefficient of the solvent employed; that time at acidities below pH 4.0 must be kept at a minimum; and that wherever possible continuous extraction and centrifuging procedures should be used.

In this work it was also demonstrated that impure penicillin was less stable than pure penicillin, and that 832 penicillin (primarily penicillin G) was substantially more stable than the 1249 variety (primarily penicillin F). The investigation thus supplied quantitative data in support of phenomena already encountered by workers in the field.

English investigators early recognized that penicillin was rapidly destroyed by an enzyme or enzymes of bacterial origin, and for this reason strict aseptic precautions had to be observed in the production process. In surface-culture work such contamination can prove an insufferable nuisance, but often is not critical since the invasion is generally localized and the contents of such vessels can be discarded. In tank fermentations it is a most serious problem, and overcoming it represents one of the outstanding engineering achievements of the whole penicillin development. But penicillin-destroying enzymes can be useful and are of particular merit in rendering penicillin samples inactive in connection with testing them for sterility. By a fortuitous accident one of our survey flasks, in which a mold was being tested for penicillin production, became contaminated with a strain of *Bacillus cereus* (subsequently designated B–569 in the NRRL Collection). This produced a peculiarly powerful penicillinase, which, when concentrated and dried in vacuo, will easily destroy one hundred times its weight of crystalline penicillin in three hours at pH 7.0 and 30° C. Methods for producing the enzyme were worked out and its characteristics were defined. The possible application of this enzyme in the assay of penicillin has been suggested.

THE CHEMISTRY OF PENICILLIN

As soon as it had been demonstrated that the commercial production of penicillin was feasible, it became evident that chemical studies were required for the most effective continuance of the penicillin program. Accordingly, a group of chemists trained in the isolation of natural products was assembled and assigned first to the urgent problem of developing a practical method for the recovery of penicillin from culture liquors. A solvent-extraction method had already been proposed by the English workers, but it offered the serious difficulty that extremely stable emulsions were produced. In industry, it became necessary, in order to break the emulsions, to install supercentrifuges, which were expensive and hard to procure. There was, then, a real need for a different method of removing penicillin from culture liquors.

It was known that penicillin could be adsorbed from solution by activated carbon, but heavy losses, due to inactivation of penicillin and inability to elute it from the carbon, militated against its industrial application. However, by proper choice of pH for the adsorption and the use of solvents, such as amyl acetate, for the elution, Dr. J. L. Wachtel of this laboratory was able to develop a recovery method that was used commercially until better methods became available.[3]

Although the early commercial production of penicillin proceeded at a gratifying rate, the military needs for the drug were so heavy that it appeared at the time that only through synthesis could these demands be met. Toward this end, the chemical group attacked the problem of the isolation of the pure penicillin required for a determination of the chemical constitution.

In common with the experience in other laboratories, much difficulty was encountered in the purification of penicillin because of its great instability. This lability prompted us to attempt conversion of our highly purified, but amorphous, penicillin fractions to a derivative having more attractive properties. Since nothing was known at the time about the nature of the reactive grouping in penicillin, we were led to the selection of benzylamine as a reagent because of its versatility in the formation of derivatives of acids, anhydrides, aldehydes, esters, lactones, ethylene oxides, azlactones, and so forth. This proved to be a fortunate first choice, for there resulted in high yield a beautifully crystalline product with good properties, the first crystalline derivative of penicillin. Studies on the structure of this compound showed it to be derived from the penicillin F investigated by the British workers.

At about this time, the isolation of crystalline penicillin G sodium salt was reported by the Squibb Institute for Medical Research, and the corresponding benzylamine derivative of this penicillin was prepared. Aside from their value for the characterization of the penicillins, the benzylamine derivatives proved to be very useful to us and to the Merck investigators for the elucidation of the structure of penicillin, since the special derivative just mentioned proved to be ideally suited for the location of the 16th or labile carbon atom of penicillin.

By the end of 1943, the structure of penicillin was believed to be known with enough certainty to justify, in all the collaborating laboratories, an extensive program having as its object the synthesis of penicillin. Indeed, the prospect of synthesis was at that time a bright one. Accordingly, work on this problem was started in this laboratory in January 1944 under OSRD (CMR) contract, but by July of that year the hope of successful completion of the problem in time to aid the war effort became so remote that the project was discontinued. This decision proved to be sound in view of the

[3] The details of the process are to be found in the specifications of U. S. Patent No. 2,399,840, granted May 7, 1946.

striking increase in the microbiologic production of penicillin in the meantime and the subsequent failure of others to develop a synthesis of any industrial promise. It should be mentioned that the chemical work in this laboratory did result in the development of a very satisfactory method for the preparation of penaldic acids and their derivatives, which may find future use in the synthesis of penicillin.

One of the major contributions of the chemists to the penicillin program has been the isolation and characterization of the various members of the penicillin family. Drs. Coghill, Stodola, and Wachtel contributed to this phase of the work by isolating, for the first time, penicillin X, the most interesting of the penicillins from the chemical point of view.

In most of the plants first erected in this country for penicillin production, the surface method of fermentation, now obsolete, was employed. One of the steps in the purification process most commonly used was the extraction of acidified penicillin solutions with chloroform. It was noted independently at the Cutter Laboratories, the Cheplin Biological Laboratories, and the Ben Venue Laboratories that at times as much as 30 per cent of the activity remained in the aqueous phase even after repeated extraction with this solvent. Since all the penicillins known at that time — namely, penicillins F, dihydro F and G, and flavicidin — were readily extractable with chloroform, it was evident that this chloroform-insoluble material, known as "factor X," was a penicillin with unusual properties, if, indeed, it was a penicillin at all. Because a penicillin so different in physical properties could have interesting therapeutic properties, we obtained from the Cutter and Chaplin laboratories a supply of the crude antibiotic with the view of isolating it in the pure state. By the use of a silica gel column followed by a Brockmann's alumina column, it was possible to isolate a pure crystalline compound, $C_{10}H_{17}N_2O_5SNa$, which was designated as penicillin X. It was soon established that penicillin X differed from penicillin G only in having a p-hydroxy group in the benzene ring.

The isolation of penicillin X created considerable interest, because it is the only one of the penicillins in which any significant chemical modification of the molecule is possible. Halogen derivatives could be prepared with activities higher than that of penicillin X itself. Of more importance, however, was the observation that a wide variety of diazotized amines could be coupled with penicillin X to give azopenicillins, some of which show very high activity and some promise of clinical value. The details of this work will appear in a chapter on "The Modified Natural Penicillins," by Coghill, Stodola, and Wachtel, in a forthcoming monograph on the chemistry of penicillin.

In addition to carrying on the chemical studies already mentioned, the chemical investigators at the Northern Regional Research Laboratory were called on to isolate the commercially important penicillins F, G, X, and K in a state of high purity and to determine with precision their physical con-

stants, so that these penicillins could be distributed as standards to investigators throughout the world. The multiplicity of penicillins had made it evident at an early date that only by the isolation and characterization of the pure penicillins could any progress be made in the assay and chemical testing of the various penicillins. Of the many samples distributed, the International Standard prepared by Stodola and Wachtel is of most interest. Samples of penicillin G sodium salt, contributed by manufacturers in this country and England, were pooled and recrystallized, and the physical constants were accurately determined by the members of the Analytical and Physical Division of the Northern Laboratory. Ten grams of the pure salt were sent in 2-gm. lots, during April and May 1945, to Sir Percival Hartley, Director of Biological Standards, British Medical Research Council, to be kept, under conditions ensuring its safety and permanence, at the National Institute for Medical Research, London, from which center it could be distributed under the direction of the Health Organization of the League of Nations or such international peace organizations as should succeed it.

SUMMARY

Research on penicillin production at the Northern Regional Research Laboratory has been paralleled by investigations of a somewhat similar nature carried on in many other laboratories. Current production of this drug is, of course, based on the knowledge gathered in the sum of such studies. This laboratory, working with the Committee on Medical Research, however, takes justifiable pride in having supplied much of this essential information. Briefly summarized, the principal contributions of this laboratory may be listed as follows:

(1) Development of the corn steep-lactose media for penicillin production.

(2) Demonstration that penicillin could be produced in submerged culture.

(3) Production of increased penicillin yields by the addition of phenylacetic acid.

(4) Improvement and refinement of assay methods.

(5) Isolation of new penicillin-producing molds and the development of superior strains capable of greatly increased production.

(6) Distribution of proved cultures to industries and research laboratories investigating penicillin.

(7) Definition of certain factors affecting the stability of penicillin.

(8) Development of a carbon process for penicillin recovery.

(9) Preparation of crystalline benzylamine derivatives of the penicillins.

(10) Isolation and characterization of penicillin X.

(11) Preparation of the azopenicillins and other derivatives.

(12) Preparation and characterization of the International Penicillin Standard.

Part Ten: Sensory Devices

CHAPTER LIV

SENSORY DEVICES

GEORGE W. CORNER

THE Committee on Sensory Devices, one of the latest and most unusual among the many enterprises of the Office of Scientific Research and Development, was created to find out what new aid science could bring to men who had lost their eyesight in the war.

The idea of such an undertaking originated with Dr. Vannevar Bush early in the war period. His attention had already been called to the unsatisfactory situation then existing with respect to artificial limbs and other mechanical aids for defective function of the arms and legs. Reflecting on the possibility of improving these devices by the application of modern scientific and engineering methods, Dr. Bush went on to consider the feasibility of similar aid to the blinded veterans of the war.

There were at this time conversations between Dr. Bush and other scientific men, notably Dr. Vladimir K. Zworykin of the Radio Corporation of America. The latter had himself been thinking about various devices for the aid of the blind, including especially one for converting printed matter into some sort of sensory stimulation other than visual — in other words, a reading machine for the blind. One of Dr. Bush's advisers had suggested even more far-reaching ideas, such as direct stimulation of the optic nerve behind a damaged eye, or of the visual region of the brain itself, by modulated electrical impulses representing the visible world.

Late in 1943, when the ending of work on military weapons was foreseeable and attention was naturally turning toward reparative work, Dr. Bush determined to set up a committee in the Office of Scientific Research and Development to explore these projects. Obviously, there were no scientific men already prepared by direct experience to lead work in this undeveloped and almost unheard-of field. The membership was presumably chosen with the thought that the projected studies called primarily for

I

knowledge of the human organism, combined with a high order of respect for research and engineering. The new committee consisted of George W. Corner, M.D., Director of the Department of Embryology of the Carnegie Institution of Washington (Chairman); Henry A. Barton, Ph.D., Director of the American Institute of Physics; A. J. Carlson, Ph.D., Professor Emeritus of Physiology, University of Chicago; Wallace O. Fenn, Ph.D., Professor of Physiology, University of Rochester; Stacy R. Guild, Ph.D., Otological Research Laboratory, Johns Hopkins Hospital; and Karl S. Lashley, Ph.D., Director of the Yerkes Laboratories of Primate Biology.

PLANS FOR RESEARCH

At its first meeting, the Committee on Sensory Devices developed a set of more or less definite ideas for a reading machine and for a guidance device — a direction-finder or obstacle-detector to be carried by the blind man when walking. The former of these would be of distinct value to the blind because, if at all successful, it would open a far wider range of reading than can be reached through braille. The latter is not easily learned, and because the printing of books in braille is expensive the range of available literature is relatively narrow. Everything a blind man learns from books, whether through braille, from phonograph records, or through a reader, comes to him through someone else's special choice or effort. Only if he were given the power to read ordinary print could he exercise the power possessed by sighted people of ranging over the whole field of literature at his own pace and by his own choice. To make this possible is the aim of those who have dreamed of a reading device.

The value of the other one of the Committee's main projects, the guidance device, needs little explanation. It is obvious that a blind man could well use an exploring device, to be carried about as a seeing person carries a flashlight in dark places. The device would convey information about objects in the visible world by acting through the user's sense of hearing or of touch. Such an instrument, even if it were beamed on only one spot at any one moment, could at least give warning of obstacles, find wanted objects, and indicate pathways. Its use might even point the way ultimately to something approaching pictorial representation of the external world.

A device of this kind, in its simplest form, operates by picking up energy from the objects explored, just as the eye picks up reflected light. One possible type of guidance device, in fact, is a mechanical eye that receives light rays and converts them, by means of photoelectric cell, into sound waves heard by the blind man through an earphone. The Committee's plans included the study of this kind of apparatus, but it also considered other forms of energy besides that of visible light. The use of ultraviolet waves and, at the other end of the spectrum, the "radar" or ultra-short radio waves is

scarcely practicable for technical reasons. Energy in the form of supersonic waves is, however, worth serious consideration and has in its favor the strong argument that Nature has already applied it successfully. Recent studies by biologists have proved that bats avoid obstacles, when flying in the dark, by uttering supersonic cries, inaudible to human ears but receivable by the bat when reflected from objects in its path.

The Committee initiated and maintained liaison with the several military and civilian agencies concerned with the care of the blind in this country and with St. Dunstan's Institution in England. By way of breaking the ice with one of these agencies, the Army General Hospital at Valley Forge, the Committee arranged for the design and construction of a simple and very effective device by which blind soldiers could take part in the sport of bowling, locating the pins by a head-borne, directionally selective photoelectric cell that detected a light over the end of the alley. The principle of this device, although useful for the special need, is of course too limited in its nature for general application to the problems of direction-finding.

EXPERIMENTAL PROGRAM

The Committee decided to conduct its work with the aid of a central laboratory, employed under contract to make preliminary studies, integrate the work done by other contractors, and conduct theoretical and practical tests of devices as they were developed. The Haskins Laboratories of New York City were selected for this purpose.

APPLIED PSYCHOLOGY

The Committee was aware from the first that its main problems would not be those of physics and instrumental design. Difficult as the mechanical and electronic work might be, the engineers could be expected to make progress in this direction. The critical question is how much a blind man can learn about the visible world through instruments that stimulate his hearing or his sense of touch. How far can a buzz in his ears or a tingle on his skin be made to give him knowledge of a printed word or of an obstacle in his path? These machines will have to speak in codes that can be learned and that can be made to convey images of useful quality. In short, much of the problem lies in the field of applied psychology.

The staff of the central laboratory therefore undertook as its primary duty the psychological analysis of intelligibility and learnability of audible signals of the type such instruments might produce. Adequate equipment for the purpose was assembled and used in an extensive study, the results of which were made available to the industrial contractors. The Columbia Broadcasting Company designed and constructed one of the complex instru-

ments employed in this investigation, an apparatus for studying the theory of guidance devices and the characteristics and utilization of the signals producible by them. This is essentially an apparatus for recording and analyzing signals produced under controlled conditions simulating the action of guidance devices. It is used to test the learnability and usability of such signals.

When devices progress beyond the laboratory stage and begin to be tested in actual use, psychological problems on a high level can be attacked — those that concern the more complete integration of the signals into a mental picture of the objects scanned. Thus, the progress of instrumental design and of the users' psychological processes may be expected to advance side by side.

The Reading Device

It is too early at this time (February 1946) for a definitive report on the progress of the reading device. The Radio Corporation of America has developed a most ingenious instrument, which is undergoing tests by the Committee at its central laboratory and by the Navy at its Medical Research Unit at Bethesda, Maryland. It is fully portable, consisting only of a stylus-like scanner to be held in the hand, a battery case, containing also the amplifier, and an earphone. It has already proved usable at the rate of at least a few words per minute.

Dr. Zworykin and his colleagues began their work on the basis of the optophone, constructed a generation ago by Fournier d'Albe. This device was operated by means of a scanning disk, having five small holes arranged close together radially near its edge, which was revolved over an enlarged image of the printed matter to be read. Each of the holes was connected, by means of a selenium cell and a suitable circuit, to a device for producing a musical sound. The five sounds thus produced were of different pitch. As the black image of a letter of the alphabet was traversed by the five holes, whatever black area was under a given hole caused the production of its corresponding musical tone. For example, as the capital T was scanned, the top hole produced its musical sound during the whole traverse, and all five holes sounded when they crossed at one time the vertical bar of the T. In this way a twittering musical sequence was produced, which the blind were expected to interpret as language. In order to secure accurate scanning, the book was held on a rigid guide, operated by a motor. The device was well contrived from an engineering viewpoint, but it was difficult to operate and the code was too complicated for ready learning.

Dr. Zworykin and his colleagues have modified the optophone principle. In the first place, the scanner, held in the reader's hand, projects a very narrow beam so as to make a point of light on the paper. The beam is

rapidly moved up and down, so that as the reader's hand moves the stylus from left to right across the letters, the moving dot of light on the paper traverses it vertically several times. The photoelectric cell and the circuit are so arranged that when the dot of light strikes a black part of the letter near the top of its vertical traverse, a musical note of low pitch is produced in the earphone; when the dot of light is at the lower end of its traverse, a musical sound of higher pitch is produced; intermediate points on the letters produce intermediate sounds. As a result of this variation of tones, the reader is able to keep his stylus more or less accurately on the line by holding it at a level that produces an average tone. As he passes vertically from line to line he finds a silent band in the white space between the lines. The code produces sounds very much like those of the optophone but is somewhat simpler.

Guidance Devices

Three projects for guidance devices have also reached the stage of first testing in the laboratory. The Brush Development Company brought to the Committee's program a good deal of experience with supersonic energy and had even considered, since October 1941, the use of supersonic waves for guiding the blind. Under contract with the Committee, the company pursued this line of investigation and produced a fully portable instrument consisting of battery case and amplifier, an ear plug, and a combined sender-receiver small enough to be used in the hand like a flashlight, though not yet reduced to optimum size. This achievement has demonstrated the possibility of producing the necessary energy with a portable instrument. In preliminary tests at the central laboratory, the sensitivity of the apparatus has proved adequate, and its signals give intelligible information about simple obstacles.

The Stromberg-Carlson Company has made extensive studies for the Committee of the general properties of supersonic waves under conditions such as will exist in guidance devices for the blind. Their studies will undoubtedly be of great value in planning refinements and new designs, if the supersonic type of instrument proves practically useful. Their engineers have built a semiportable instrument, which has been actively studied at the central laboratory for several months, and have now constructed a fully portable device, which is nearly ready for test.

Both the foregoing devices depend on the principle of supersonic rays emitted in short pulses many times per minute. These pulses are reflected from objects in front of the user, and on returning to the device that he holds in his hand are received and heterodyned against the outgoing pulses. The result is a sound that varies in pitch and intensity according to the distance of the object from which it is reflected. By paying careful attention to these

characteristics of the sound the user is able not only to detect the presence of a reflecting object in front of him, but also to judge its distance with a fair degree of accuracy.

A third group of workers, the research staff of the Hoover Company, undertook to produce an instrument equipped to generate supersonic waves mechanically. Such an instrument would obviate the need of battery power and presumably be lighter and more compact than the electronic sets. The task has proved quite difficult because of lack of fundamental data, but the instrument is nearly ready for laboratory testing. It contains a gating device that permits the returning signals to actuate the earphone only when the apparatus is adjusted for a specific distance. The user, carrying the device in his hand, is able by squeezing a handle to change the adjustment and thus to gauge the distance of the reflecting objects quite accurately by the intensity of the sound received.

During the course of these developments the Army Signal Corps asked the Committee for co-operation in the development of guidance devices. It was agreed that the Signal Corps laboratories should undertake especially the development of the devices employing visible light, thus dividing with the Committee the exploration of possible types of instrumentation. At the time of this report, the Signal Corps has developed an experimental instrument, which is ready for preliminary tests at the central laboratory of the Committee. It depends on the principle of emitting a beam of light, receiving it when reflected, and measuring its intensity by means of an ordinary photometer, which is read by a sound produced in the earphone. The beam of light is interrupted so that the user hears a series of small bursts of sound in his ear. All the devices for ranging, involving both supersonic rays and visible light, could of course be made to convey information to the user by touch rather than by hearing, and the Committee is prepared to find this a more advantageous method from the user's standpoint. For the present, however, the design and construction of the receiver is considerably easier when the sense of hearing is used, because earphones are commercially obtainable and extremely easy to adapt to experimental devices.

Optical Devices for Reduced Visual Acuity

The Committee's attention was called to the needs of veterans who suffer, not from total blindness, but from greatly diminished visual acuity due to wounds or disease. In civilian life also there are many thousands of persons with low visual acuity who can read only if provided with magnifying lenses. The so-called telescopic eyeglasses have long been in use, but for various reasons they do not fill the needs of a large proportion of such persons. In June 1945, a contract was made with the Dartmouth Eye Institute at Hanover, New Hampshire, to study the problem of improved optical devices.

The contract resulted in the preparation of a careful survey-report on the problem, accompanied by recommendations that improved reading glasses and improved projection devices for enlarging reading matter be developed. Work on the design of a reading glass has been approved by the Committee and is under way.

SOUND RECORDING FOR THE BLIND

The Committee has also been much interested in the use of phonographic sound recording for the blind. Disk records of the conventional type, modified only as to speed of revolution and number of lines per inch, have been in use as the so-called "Talking Book" for a dozen years. There have, however, been great advances in the art of sound-recording during the war. The Committee undertook to inform itself concerning the present status of the Talking Book project and the future needs and possibilities in this field. A careful survey was made with the help of the Library of Congress, which operates the government's Talking Book Service, and in the spring of 1945 a report was distributed to interested persons. The problem is complicated by the lack of any disinterested group suitably placed and equipped to develop improvements on a large scale.

Members of the Committee have taken part in several conferences with groups interested in the improvement and extension of the Talking Book program, and have arranged for preliminary tests of certain methods and apparatus known to the industry and thought to have possible application in this field. The Committee's studies and numerous consultations with the Library of Congress, the Army Rehabilitation Center for the Blind, and the phonograph industry have stimulated interest and activity among these groups. The Chairman has represented the Committee on Sensory Devices in a special committee being formed by the Library of Congress and the industry, to plan for new reproducers of the standard disks. It seems probable that the existing agencies and the industrial laboratory experts can work out the problem without further intervention by the Committee.

SUMMARY

In summary, the Committee on Sensory Devices was assigned the difficult but potentially beneficent task of developing scientific aids for the blinded veterans of the war. During the two years at its disposal (1944 and 1945) it has been able to isolate two promising ideas, the reading machine and the guidance device, and to carry them to partial development sufficient for laboratory testing.

It has thoroughly explored the needs in two other fields (phonographic recording of literature for the blind and optical aids for subnormal vision)

and has begun development of the latter. Its work aroused sufficient interest so that at the termination of the Committee's relations with the Office of Scientific Research and Development in November 1945, the support of its contractual work was assumed by the Surgeon General's Office of the Army. At the time of writing all the Committee's lines of investigation are still under way and all but one of its original contractors are still at work.

BIBLIOGRAPHY

Publications by Investigators on OSRD/CMR Contracts

MEDICINE

Infectious Diseases

Anthrax

MURPHY, F. D., LaBOCCETTA, A. C., AND LOCKWOOD, J. S. Treatment of human anthrax with penicillin: Report of three cases. *J.A.M.A.*, 1944, *126*: 948–950.

Bacillary Dysentery

BINKLEY, F., GOEBEL, W. F., AND PERLMAN, E. Studies on the Flexner group of dysentery bacilli. II. The chemical degradation of the specific antigen of Type Z Shigella paradysenteriae (Flexner). *J. Exper. Med.*, 1945, *81*: 331–347.

COOPER, M. L., TEPPER, J., AND KELLER, H. M. Active immunization of children and animals with Shigella paradysenteriae Flexner. *J. Bact.*, 1944, *47*: 477.

DOAK, B. W., HALBERT, S. P., SMOLENS, J., AND MUDD, S. A comparison of rabbit and mouse antisera to Shigella paradysenteriae. *J. Immunol.*, 1946, *52*: 113–120.

DUBOS, R. J., HOBERMAN, H. D., AND PIERCE, C. Some factors affecting the toxicity of cultures of Shigella dysenteriae. *Proc. Nat. Acad. Sc.*, 1942, *28*: 453–458.

DUBOS, R. J., STRAUS, J. H., AND PIERCE, C. The multiplication of bacteriophage in vivo and its protective effect against an experimental infection with Shigella dysenteriae. *J. Exper. Med.*, 1943, *78*: 161–168.

EHRICH, W. E., HALBERT, S. P., MERTENS, E., AND MUDD, S. Mechanism of the augmenting acting of mineral oil on antibody production. Tissue reactions and antibody response to dysentery vaccine in saline, and in saline-lanolin-mineral oil emulsion. *J. Exper. Med.*, 1945, *82*: 343–360.

GOEBEL, W. F., BINKLEY, F., AND PERLMAN, E. Studies on the Flexner group of dysentery bacilli. I. The specific antigens of Shigella paradysenteriae (Flexner). *J. Exper. Med.*, 1945, *81*: 315–330.

GOEBEL, W. F., PERLMAN, E., AND BINKLEY, F. Antibody response in man to injection of the specific antigen of Type V Shigella paradysenteriae. *Science*, 1944, *99*: 412–413.

HALBERT, S. P., MUDD, S., AND SMOLENS, J. The antigenicity of Shigella paradysenteriae (Flexner) in saline-in-mineral-oil emulsion. *Proc. Soc. Exper. Biol. & Med.*, 1945, *60*: 17–19.

HALBERT, S. P., SMOLENS, J., AND MUDD, S. Reduced acute toxicity of antigens in saline-in-mineral-oil emulsion. *J. Immunol.*, 1945, *51*: 39–43.

HARDY, A. V. Studies of the acute diarrheal diseases. XC. Further cultural observations on the relative efficacy of sulfonamides in Shigella infections. *Pub. Health Rep.*, 1945, *60*: 1037–1042.

HARDY, A. V., BURNS, W., AND DeCAPITO, T. Studies of acute diarrheal diseases. XA. Cultural observations on the relative efficacy of sulfonamides in Shigella dysenteriae infections. *Pub. Health Rep.*, 1943, *58*: 689–693.

HARDY, A. V., AND CUMMINS, S. D. Studies of the acute diarrheal diseases. XB. A preliminary note on the clinical response to sulfadiazine therapy. *Pub. Health Rep.*, 1943, *58*: 693–696.

MOORE, F., KESSEL, J. F., SIMONSEN, D. G., AND MARMORSTON, J. Experimental basis of sulfonamide therapy in bacillary dysentery. *J. Infect. Dis.*, 1946, *78*: 25–31.

PERLMAN, E., BINKLEY, F., AND GOEBEL, W. F. Studies on the Flexner group of dysentery bacilli. III. Antibody response in man following the administration of the specific antigen of Type V Shigella paradysenteriae (Flexner). *J. Exper. Med.*, 1945, *81*: 349–358.

PERLMAN, E., AND WEIL, A. J. Relationship between precipitative and protective antibodies of Type III Shigella paradysenteriae (Flexner) immune serum. *Proc. Soc. Exper. Biol. & Med.*, 1945, *60*: 58–60.

SMOLENS, J., HALBERT, S. P., MUDD, S., DOAK, B. W., AND GONZALEZ, L. M. Studies with the somatic antigen of Shigella paradysenteriae (Flexner). *J. Immunol.*, 1946, *52*: 41–58.

TREFFERS, H. P. The detoxification by acetylation of soluble antigens from Shigella dysenteriae (Shiga) and E. typhosa. *Science,* 1946, *103:* 387–389.

WATT, J., AND CUMMINS, S. D. Studies of the acute diarrheal diseases. XD. Further studies on the relative efficacy of sulfonamides in shigellosis. *Pub. Health Rep.,* 1945, *60*: 1355–1361.

Cholera

BURROWS, W. The endotoxin of the cholera vibrio: Isolation and properties. *Proc. Soc. Exper. Biol. & Med.*, 1944, *57:* 306–308.

· BURROWS, W., MATHER, A. N., WAGNER, S. M., AND McGANN, V. G. The endotoxin of the cholera vibrio: Immunological properties. *Proc. Soc. Exper. Biol. & Med.*, 1944, *57:* 308–311.

BURROWS, W., WAGNER, S. M., AND MATHER, A. N. The endotoxin of the cholera vibrio: Action on living semipermeable membranes. *Proc. Soc. Exper. Biol. & Med.*, 1944, *57:* 311–314.

EISELE, C. W., McCULLOUGH, N. B., BEAL, G. A., AND BURROWS, W. Development of brucella agglutinins in humans following vaccination for cholera. *Proc. Soc. Exper. Biol. & Med.*, 1946, *61:* 89–91.

JENNINGS, R. K. Starch digestion by Vibrio cholerae in strongly aerated media. *J. Bact.*, 1945, *49:* 163–166.

JENNINGS, R. K., AND LINTON, R. W. The biochemistry of Vibrio cholerae. II. The influence of environmental factors on growth. *Arch. Biochem.*, 1944, *3:* 429–438.

JENNINGS, R. K., AND LINTON, R. W. The biochemistry of Vibrio cholerae. III. Acid regulation by means of the carbon-dioxide-bicarbonate buffering system. *Arch. Biochem.,* 1944, *4:* 311–318.

JENNINGS, R. K., AND LINTON, R. W. Production and properties of BRF direct cholera vaccine. *J. Franklin Inst.,* 1944, *238:* 65–70.

LINTON, R. W., AND JENNINGS, R. K. The biochemistry of Vibrio cholerae. I. Growth methods. *Arch. Biochem.*, 1944, *3:* 419–427.

Encephalitis

DUFFY, C. E., AND STANLEY, W. M. Studies on the biochemical, biophysical, and immunogenic properties of Japanese B type encephalitis virus and vaccines. *J. Exper. Med.,* 1945, *82:* 385–410.

HAMMON, W. McD., AND REEVES, W. C. Recent advances in the epidemiology of the arthropod-borne virus encephalitides including certain exotic types. *Am. J. Pub. Health,* 1945, *35:* 994–1004.

HAMMON, W. McD., AND REEVES, W. C. Laboratory transmission of St. Louis encephalitis virus by three genera of mosquitoes. *J. Exper. Med.,* 1943, *78:* 241–253.

HAMMON, W. McD., AND REEVES, W. C. Laboratory transmission of Western equine encephalomyelitis virus by mosquitoes of the genera Culex and Culiseta. *J. Exper. Med.,* 1943, *78:* 425–434.

HAMMON, W. McD., REEVES, W. C., BENNER, S. R., AND BROOKMAN, B. Human encephalitis in the Yakima Valley, Washington, 1942, with forty-nine virus isolations (Western equine and St. Louis types) from mosquitoes. *J.A.M.A.,* 1945, *128:* 1133–1139.

HAMMON, W. McD., REEVES, W. C., AND GALINDO, P. Epizoology of Western equine type encephalomyelitis: Eastern Nebraska field survey of 1943 with isolation of the virus from mosquitoes. *Am. J. Vet. Research,* 1945, *6:* 145–148.

HAMMON, W. McD., REEVES, W. C., IRONS, J. V. Survey of arthropod-borne virus encephalitis in Texas with particular reference to Lower Rio Grande Valley in 1942. *Texas Rep. Biol. & Med.,* 1944, *2:* 366–375.

REEVES, W. C., AND HAMMON, W. McD. Feeding habits of the proven and possible mosquito vectors of Western equine and St. Louis encephalitis in the Yakima Valley, Washington. *Am. J. Trop. Med.,* 1944, *24:* 131–134.

Endocarditis, Subacute Bacterial

FLIPPIN, H. F., MAYOCK, R. L., MURPHY, F. D., AND WOLFERTH, C. C. Penicillin in the treatment of subacute bacterial endocarditis; a preliminary report on twenty cases treated over one year ago. *J.A.M.A.,* 1945, *129:* 841–843.

GLASER, R. J., SMITH, R. O., HARFORD, C. G., AND WOOD, W. B. JR. The treatment of bacterial endocarditis with penicillin. *J. Lab. & Clin. Med.,* 1946, *31:* 291–312.

Food Poisoning

EDWARDS, P. R. A paracolonlike bacillus isolated from colitis in an infant. *J. Bact.* 1945, *49:* 513.

EDWARDS, P. R. Salmonella pomona and Salmonella champaign; two hitherto undescribed types isolated from fowls. *Proc. Soc. Exper. Biol. & Med.,* 1945, *58:* 291–292.

EDWARDS, P. R. An outbreak of Salmonella pullorum infection in canaries. *J. Am. Vet. M. A.,* 1945, *107:* 245.

EDWARDS, P. R. Form variation in group C Salmonella strains. *Proc. Soc. Exper. Biol. & Med.,* 1945, *59:* 49–52.

EDWARDS, P. R., AND MORAN, A. B. Salmonella cultures which resemble the Sendai type. *J. Bact.,* 1945, *50:* 257–260.

EDWARDS, P. R., AND WEST, M. G. Phase variation of Andrewes in a coliform bacterium. *J. Infect. Dis.,* 1945, *77:* 185–186.

MORAN, A. B., AND EDWARDS, P. R. A new Salmonella type: Salmonella pensacola. *Proc. Soc. Exper. Biol. & Med.*, 1945, *59:* 52–54.

WEST, M. G., DOLL, E. R., AND EDWARDS, P. R. Inhibition of Salmonella cultures by streptomycin. *Proc. Soc. Exper. Biol & Med.*, 1945, *60:* 363:364.

Gas Gangrene — see SURGERY, Wounds and Burns

Influenza

HENLE, W., AND HENLE, G. Interference between inactive and active viruses of influenza. I. The incidental occurrence and artificial induction of the phenomenon. *Am. J. M. Sc.,* 1944, *207:* 705–717.

HENLE, W., AND HENLE, G. Interference between inactive and active viruses of influenza. II. Factors influencing the phenomenon. *Am. J. M. Sc.,* 1944, *207:* 717–733.

HENLE, W., AND HENLE, G. Interference between inactive and active viruses of influenza. III. Cross-interference between various related and unrelated viruses. *Am. J. M. Sc.,* 1945, *210:* 362–369.

HENLE, G., AND HENLE, W. Interference between inactive and active viruses of influenza. IV. The nature of the interfering agent. *Am. J. M. Sc.,* 1945, *210:* 369–374.

HENLE, W., AND HENLE, G. The toxicity of influenza viruses. *Science,* 1945, *102:* 398–400.

HENLE, W., AND HENLE, G. Effect of adjuvants on vaccination of human beings against influenza. *Proc. Soc. Exper. Biol. & Med.,* 1945, *59:* 179–181.

HENLE, G., AND HENLE, W. Neurological signs in mice following intracerebral inoculation of influenza viruses. *Science,* 1944, *100:* 410–411.

HENLE, W., HENLE, G., HAMPIL, B., MARIS, E. P., AND STOKES, J. JR. Experiments on vaccination of human beings against epidemic influenza. *J. Immunol.,* 1946, *53:* 75–93.

HENLE, W., HENLE, G., STOKES, J. JR., AND MARIS, E. P. Experimental exposure of human subjects to viruses of influenza. *J. Immunol.,* 1946, *52:* 145–165.

KNIGHT, C. A. Precipitin reactions of highly purified influenza viruses and related materials. *J. Exper. Med.,* 1946, *83:* 281–294.

KNIGHT, C. A. The preparation of highly purified PR8 influenza virus from infected mouse lungs. *J. Exper. Med.,* 1946, *83:* 11–24.

KNIGHT, C. A. Titration of influenza virus in chick embryos. *J. Exper. Med.,* 1944, *79:* 487–495.

KNIGHT, C. A. The stability of influenza virus in the presence of salts. *J. Exper. Med.,* 1944, *79:* 285–290.

KNIGHT, C. A. A sedimentable component of allantoic fluid and its relationship to influenza viruses. *J. Exper. Med.,* 1944, *80:* 83–100.

KNIGHT, C. A., AND STANLEY, W. M. The effect of some chemicals on purified influenza virus. *J. Exper. Med.,* 1944, *79:* 291–300.

LAUFFER, M. A., AND CARNELLY, H. L. Thermal destruction of influenza A virus hemagglutinin. I. The kinetic process. *Arch. Biochem.,* 1945, *8:* 265–274.

LAUFFER, M. A., AND MILLER, G. L. The sedimentation rate of the biological activities of influenza A virus. *J. Exper. Med.,* 1944, *80:* 521–529.

LAUFFER, M. A., AND MILLER, G. L. The mouse infectivity titration of influenza virus. *J. Exper. Med.,* 1944, *79*: 197–203.

LAUFFER, M. A., AND SCOTT, E. M. Thermal destruction of influenza A virus hemagglutinin. II. The effect of pH. *Arch. Biochem.,* 1946, *9*: 75–80.

LAUFFER, M. A., AND STANLEY, W. M. Biophysical properties of preparations of PR8 influenza virus. *J. Exper. Med.,* 1944, *80*: 531–548.

MILLER, G. L. A study of conditions for the optimum production of PR8 influenza virus in chick embryos. *J. Exper. Med.,* 1944, *79*: 173–183.

MILLER, G. L. Influence of pH and of certain other conditions on the stability of the infectivity and red cell agglutinating activity of influenza virus. *J. Exper. Med.,* 1944, *80*: 507–520.

MILLER, G. L., LAUFFER, M. A., AND STANLEY, W. M. Electrophoretic studies on PR8 influenza virus. *J. Exper. Med.,* 1944, *80*: 549–559.

MILLER, G. L., AND STANLEY, W. M. Quantitative aspects of the red blood cell agglutination test for influenza virus. *J. Exper. Med.,* 1944, *79*: 185–195.

STANLEY, W. M. The size of influenza virus. *J. Exper. Med.,* 1944, *79*: 267–283.

STANLEY, W. M. The precipitation of purified concentrated influenza virus and vaccine on calcium phosphate. *Science,* 1945, *101*: 332–335.

STANLEY, W. M. The preparation and properties of influenza virus vaccines concentrated and purified by differential centrifugation. *J. Exper. Med.,* 1945, *81*: 193–218.

STANLEY, W. M. An evaluation of methods for the concentration and purification of influenza virus. *J. Exper. Med.,* 1944, *79*: 255–266.

STOKES, J. JR., HENLE, W., HENLE, G., AND MARIS, E. P. Studies in human vaccination against influenza A and B by the subcutaneous and inhalation methods. *Tr. A. Am. Physicians,* 1944, *58*: 192–195.

Hemolytic Streptococcal Infection

ELLIOTT, S. D. A proteolytic enzyme produced by group A streptococci with special reference to its effect on the type-specific M antigen. *J. Exper. Med.,* 1945, *81*: 573–592.

LANCEFIELD, R. C., AND STEWART, W. A. Studies on the antigenic composition of Group A hemolytic streptococci. II. The occurrence of strains in a given type containing M but no T antigen. *J. Exper. Med.,* 1944, *79*: 79–88.

RANTZ, L. A. Group A hemolytic streptococcus antibodies. III. A study of the simultaneous infection of a large number of men by a single type. *Arch. Int. Med.,* 1944, *73*: 238–240.

RANTZ, L. A., AND RANDALL, E. A modification of the technic for the determination of the antistreptolysin titer. *Proc. Soc. Exper. Biol. & Med.,* 1945, *59*: 22–25.

STEWART, W. A., LANCEFIELD, R. C., WILSON, A. T., AND SWIFT, H. F. Studies on the antigenic composition of group A hemolytic streptococci. IV. Related T but distinct M antigens in types 15, 17, 19, 23, 30, and in types 4, 24, 26, 28, 29, 46. Identification by slide agglutination. *J. Exper. Med.,* 1944, *79*: 99–114.

SWIFT, H. F., WILSON, A. T., AND LANCEFIELD, R. C. Typing group A hemolytic streptococci by M precipitin reactions in capillary pipettes. *J. Exper. Med.,* 1943, *78*: 127–133.

WATSON, R. F., AND LANCEFIELD, R. C. Studies on the antigenic composition of

group A hemolytic streptococci. III. Types with serologically identical M but distinct T antigens: Types 10 and 12. *J. Exper. Med.,* 1944, *79:* 89–98.

Measles

JANEWAY, C. A. Use of concentrated human serum gamma-globulin in the prevention and attenuation of measles. *Bull. New York Acad. Med.,* 1945, *21:* 202–222.

JANEWAY, C. A. — see PHYSIOLOGY, Shock.

ORDMAN, C. W., JENNINGS, C. G., AND JANEWAY, C. A. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XII. The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles. *J. Clin. Investigation,* 1944, *23:* 541–549.

Mumps

ENDERS, J. F. Observations on immunity in mumps. *Ann. Int. Med.,* 1943, *18:* 1015–1019.

ENDERS, J. F., COHEN, S., AND KANE, L. W. Immunity in mumps. II. The development of complement-fixing antibody and dermal hypersensitivity in human beings following mumps. *J. Exper. Med.,* 1945, *81:* 119–135.

ENDERS, J. F., KANE, L. W., COHEN, S., AND LEVENS, J. H. Immunity in mumps. I. Experiments with monkeys (Macacus mulatta). The development of complement-fixing antibody following infection and experiments on immunization by means of inactivated virus and convalescent human serum. *J. Exper. Med.,* 1945, *81:* 93–117.

Pneumonia

BUNN, P. A., McDERMOTT, W., HADLEY, S. J., AND CARTER, A. C. The treatment of pneumococcic pneumonia with orally administered penicillin. *J.A.M.A.,* 1945, *129:* 320–327.

CURNEN, E. C., AND HORSFALL, F. L. JR. Studies on pneumonia virus of mice (PVM). III. Hemagglutination by the virus; the occurrence of combination between the virus and a tissue substance. *J. Exper. Med.,* 1946, *83:* 105–132.

CURNEN, E. C., MIRICK, G. S., ZIEGLER, J. E. JR., THOMAS, L., AND HORSFALL, F. L. JR. Studies on primary atypical pneumonia. I. Clinical features and results of laboratory investigations. *J. Clin. Investigation,* 1945, *24:* 209–226.

HORSFALL, F. L. JR., AND CURNEN, E. C. Studies on pneumonia virus of mice (PVM). I. The precision of measurements in vivo of the virus and antibodies against it. *J. Exper. Med.,* 1946, *83:* 25–42.

HORSFALL, F. L. JR., AND CURNEN, E. C. Studies on pneumonia virus of mice (PVM). II. Immunological evidence of latent infection with the virus in numerous mammalian species. *J. Exper. Med.,* 1946, *83:* 43–64.

HORSFALL, F. L. JR., CURNEN, E. C., MIRICK, G. S., THOMAS, L., AND ZIEGLER, J. E. JR. A virus recovered from patients with primary atypical pneumonia. *Science,* 1943, *97:* 289–291.

THOMAS, L., CURNEN, E. C., MIRICK, G. S., ZIEGLER, J. E. JR., AND HORSFALL, F. L. JR. Complement fixation with dissimilar antigens in primary atypical pneumonia. *Proc. Soc. Exper. Biol. & Med.,* 1943, *52:* 121–125.

THOMAS, L., MIRICK, G. S., CURNEN, E. C., ZIEGLER, J. E. JR., AND HORSFALL, F. L. JR. Serological reactions with an indifferent streptococcus in primary atypical pneumonia. *Science,* 1943, *98:* 566–568.

THOMAS, L., MIRICK, G. S., CURNEN, E. C., ZIEGLER, J. E. JR., AND HORSFALL, F. L. JR. Studies on primary atypical pneumonia. II. Observations concerning the relationship of a non-hemolytic streptococcus to the disease. *J. Clin. Investigation,* 1945, *24:* 227–240.

Respiratory Tract Infections

MIRICK, G. S., THOMAS, L., CURNEN, E. C., AND HORSFALL, F. L. JR. Studies on a non-hemolytic streptococcus isolated from the respiratory tract of human beings. I. Biological characteristics of streptococcus MG. *J. Exper. Med.,* 1944, *80:* 391–406.

MIRICK, G. S., THOMAS, L., CURNEN, E. C., AND HORSFALL, F. L. JR. Studies on a non-hemolytic streptococcus isolated from the respiratory tract of human beings. II. Immunological characteristics of streptococcus MG. *J. Exper. Med.,* 1944, *80:* 407–430.

MIRICK, G. S., THOMAS, L., CURNEN, E. C., AND HORSFALL, F. L. JR. Studies on a non-hemolytic streptococcus isolated from the respiratory tract of human beings. III. Immunological relationship of streptococcus MG to Streptococcus salivarius type I. *J. Exper. Med.,* 1944, *80:* 431–440.

Tetanus Toxoid

FEENEY, R. E., MUELLER, J. H., AND MILLER, P. A. Growth requirements of Clostridium tetani. II. Factors exhausted by growth of the organism. *J. Bact.,* 1943, *46:* 559–562.

FEENEY, R. E., MUELLER, J. H., AND MILLER, P. A. Growth requirements of Clostridium tetani. III. A "synthetic" medium. *J. Bact.,* 1943, *46:* 563–571.

MUELLER, J. H., AND MILLER, P. A. Production of tetanal toxin. *J. Immunol.,* 1945, *50:* 377–384.

MUELLER, J. H., AND MILLER, P. A. Large-scale production of tetanal toxin on a peptone-free medium. *J. Immunol.,* 1943, *47:* 15–22.

MUELLER, J. H., SCHOENBACH, E. B., JEZUKAWICZ, J. J., AND MILLER, P. A. Production of tetanus toxin on peptone-free media. *J. Clin. Investigation,* 1943, *22:* 315–318.

MUELLER, J. H., SEIDMAN, L. R., AND MILLER, P. A. Antitoxin response in man to tetanus toxoids. *J. Clin. Investigation,* 1943, *22:* 325–328.

MUELLER, J. H., SEIDMAN, L. R., AND MILLER, P. A. A comparison of antigenicities of hydrolysate and peptone tetanus toxoids in the guinea pig. *J. Clin. Investigation,* 1943, *22:* 321–324.

SCHOENBACH, E. B., JEZUKAWICZ, J. J., AND MUELLER, J. H. Conversion of hydrolysate tetanus toxin to toxoid. *J. Clin. Investigation,* 1943, *22:* 319–320.

Typhus Fever

COHEN, S. S. The enzymatic degradation of thymus nucleohistone. *J. Biol. Chem.,* 1945, *158:* 255–264.

COHEN, S. S. Observations on the estimation of desoxyribose nucleic acid. *J. Biol. Chem.,* 1944, *156:* 691–702.

COHEN, S. S. The chemical alteration of a bacterial surface, with special reference to the agglutination of B. proteus OX–19. *J. Exper. Med.*, 1945, *82*: 133–142.

COHEN, S. S., AND CHARGAFF, E. Studies on the composition of Rickettsia prowazeki. *J. Biol. Chem.*, 1944, *154*: 691–704.

Air Sterilization

BIGG, E., AND JENNINGS, B. H. The introduction of glycols for air sterilization by a new vaporization method. *J. Indust. Hyg. & Toxicol.*, 1944, *26*: 307–312.

BIGG, E., JENNINGS, B. H., AND FRIED, S. The use of glycol vapors for bacterial control in large spaces. *Am. J. M. Sc.*, 1944, *207*: 361–369.

BIGG, E., JENNINGS, B. H., AND FRIED, S. Inflammability characteristics of propylene glycol and triethylene glycol in liquid and vapor form. *Am. J. M. Sc.*, 1944, *207*: 370–374.

BIGG, E., JENNINGS, B. H., AND OLSON, F. C. W. Epidemiologic observations on the use of glycol vapors for air sterilization. *Am. J. Pub. Health*, 1945, *35*: 788–798.

BIGG, E., AND MELLODY, M. Air-borne infection can be reduced with these new techniques. *Hospitals*, 1944, *18*: 29–31.

FRIED, S., BIGGS, E., AND JENNINGS, B. H. A simple analytical method for the determination of propylene and triethylene glycol. *Science*, 1943, *98*: 479–480.

JENNINGS, B. H., AND BIGG, E. Engineering problems in the use of glycol vapors for air sterilization. *Am. J. Pub. Health*, 1944, *34*: 477–483.

JENNINGS, B. H., BIGG, E., AND OLSON, F. C. W. The use of glycol vapors for air sterilization and the control of air-borne infection. *J. Heating, Piping & Air Conditioning*, 1944, Sept.: 538–545.

Detergents

MILLER, B. F., ABRAMS, R., HUBER, D. A., AND KLEIN, M. Formation of invisible, non-perceptible films on hands by cationic soaps. *Proc. Soc. Exper. Biol. & Med.*, 1943, *54*: 174–176.

MILLER, B. F., ABRAMS, R., DORFMAN, A., AND KLEIN, M. Antibacterial properties of protamine and histone. *Science*, 1942, *96*: 428–430.

Vaccines

LEVINSON, S. O., MILZER, A., SHAUGHNESSY, H. J., NEAL, J. L., AND OPPENHEIMER, F. Production of potent inactivated vaccines with ultra-violet irradiation. II. An abbreviated preliminary report on sterilization of bacteria and immunization with rabies and St. Louis encephalitis vaccines. *J.A.M.A.*, 1944, *125*: 531–532.

LEVINSON, S. O., MILZER, A., SHAUGHNESSY, H. J., NEAL, J. L., AND OPPENHEIMER, F. A new method for the production of potent inactivated vaccines with ultraviolet irradiation. II. Sterilization of bacteria and immunization with rabies and St. Louis encephalitis vaccines. *J. Immunol.*, 1945, *50*: 317–329.

MILZER, A., OPPENHEIMER, F., AND LEVINSON, S. O. Production of potent inactivated vaccines with ultraviolet irradiation. III. An abbreviated preliminary report on a completely inactivated poliomyelitis vaccine (Lansing strain virus in mice). *J.A.M.A.*, 1944, *125*: 704–705.

MILZER, A., OPPENHEIMER, F., AND LEVINSON, S. O. A new method for the production of potent inactivated vaccines with ultraviolet irradiation. III. A com-

pletely inactivated poliomyelitis vaccine with the Lansing strain in mice. *J. Immunol.*, 1945, *50:* 331–340.

Treatment of Sewage

CRAM, E. B. The effect of various treatment processes on the survival of helminth ova and protozoan cysts in sewage. *Sewage Works J.,* 1943, *15:* 1119–1138.

CRAM, E. B., AND HICKS, D. O. The effect of sludge digestion, drying and supplemental treatment on eggs of Ascaris lumbricoides. *Proc. Helminthological Soc. of Washington,* 1944, *11:* 1–9.

Venereal Diseases

Chancroid

COMBES, F. C., AND CANIZARES, O. The experimental prophylaxis of chancroid. *Am. J. Syph., Gonor., & Ven. Dis.,* 1944, *28:* 59–65.

COMBES, F. C., AND CANIZARES, O. Experimental prophylaxis of chancroid. *Arch. Dermat. & Syph.,* 1945, *51:* 237–240.

GREENBLATT, R. B., SANDERSON, E. S., KUPPERMAN, H. S., HAIR, Q., AND FRIED, P. The experimental prophylaxis of chancroid disease — II. *Am. J. Syph., Gonor., & Ven. Dis.,* 1944, *28:* 165–178.

GREENBLATT, R. B., SANDERSON, E. S., MORTARA, F., AND KUPPERMAN, H. S. Experimental prophylaxis of chancroid disease. *Am. J. Syph., Gonor., & Ven. Dis.,* 1943, *27:* 30–43.

Gonorrhea

BANG, F. B. Experimental prophylaxis of gonococcal infections. *Am. J. Syph., Gonor., & Ven. Dis.,* 1943, *27:* 716–732.

DRELL, M. J., MILLER, C. P., BOHNHOFF, M., AND MOELLER, V. Experimental gonococcal infection of the rabbit's eye. II. Course of the disease and its pathology. *J. Infect. Dis.,* 1945, *77:* 201–215.

GOULD, R. G. Glutathione as an essential growth factor for certain strains of Neisseria gonorrhoeae. *J. Biol. Chem.,* 1944, *153:* 143–150.

GOULD, R. G., KANE, L. W., AND MUELLER, J. H. On the growth requirements of Neisseria gonorrhoeae. *J. Bact.,* 1944, *47:* 287–292.

HILL, J. H., HUFFER, V., AND NELL, E. The problem of infection of animal mucosae with Neisseria gonorrhoeae. I. Modification of host resistance. *Am. J. Syph., Gonor., & Ven. Dis.,* 1945, *29:* 281–302.

MILLER, C. P. The enhancement of virulence of the gonococcus for the mouse. *Am. J. Syph., Gonor., & Ven. Dis.,* 1944, *28:* 620–626.

MILLER, C. P., AND BOHNHOFF, M. Studies on the action of penicillin. IV. Development of penicillin resistance by gonococcus. *Proc. Soc. Exper. Biol. & Med.,* 1945, *60:* 354–356.

MILLER, C. P., BOHNHOFF, M., AND MOELLER, V. Experimental gonococcal infection of the rabbit's eye. III. Treatment with prophylactic and therapeutic agents. *J. Infect. Dis.,* 1945, *77:* 216–223.

MILLER, C. P., DRELL, M. J., MOELLER, V., AND BOHNHOFF, M. Experimental gonococcal infection of the rabbit's eye. I. Method of production. *J. Infect. Dis.,* 1945, *77:* 193–200.

MILLER, C. P., MOELLER, V., AND BOHNHOFF, M. A method for production of a localized gonococcal infection in the rabbit's eye. *Proc. Soc. Exper. Biol. & Med.*, 1945, *58:* 143–146.

Syphilis

BOAK, R. A., DORN, F. L., AND CARPENTER, C. M. The effect of fever on the toxicity of mapharsen for rabbits. *Am. J. Syph., Gonor., & Ven. Dis.*, 1945, *29:* 428–431.

BOERNER, F., NEMSER, S., AND STOKES, J. H. A study of the effect of bleeding and of repeated blood donation on serologic tests for syphilis. *Am. J. M. Sc.*, 1946, *211:* 571–576.

CALLAWAY, J. L., LOWENBACH, H., NOOJIN, R. O., KUHN, B. H., AND RILEY, K. A. Electroencephalographic findings in central nervous system syphilis before and after treatment with penicillin. *J.A.M.A.*, 1945, *129:* 938–939.

CALLAWAY, J. L., NOOJIN, R. O., FLOWER, A. H. JR., KUHN, B. H., AND RILEY, K. A. The use of penicillin in the treatment of syphilis of the central nervous system. *Am. J. Syph., Gonor., & Ven. Dis.*, 1946, *30:* 110–124.

COOPER, G. R., REIN, C. R., AND BEARD, J. W. Electrophoretic analysis of kala-azar human serum. Hypergammaglobulinemia associated with seronegative reactions for syphilis. *Proc. Soc. Exper. Biol. & Med.*, 1946, *61:* 179–183.

DAVIS, B. D., KABAT, E. A., HARRIS, A., AND MOORE, D. H. The anticomplementary activity of serum gamma globulin. *J. Immunol.*, 1944, *49:* 223–233.

DAVIS, B. D., MOORE, D. H., KABAT, E. A., AND HARRIS, A. Electrophoretic, ultracentrifugal, and immunochemical studies on Wassermann antibody. *J. Immunol.*, 1945, *50:* 1–20.

EAGLE, H., HOGAN, R. B., AND FLEISCHMAN, R. The local chemical prophylaxis of experimental syphilis with phenyl arsenoxides. *A. J. Syph., Gonor., & Ven. Dis.*, 1944, *28:* 661–681.

FLEMING, W. L., AND WOLF, M. H. The relative prophylactic effectiveness against syphilis of ointments containing calomel in different particle size. *Am. J. Syph., Gonor., and Ven. Dis.*, 1946, *30:* 47–53.

GAMMON, G. D., STOKES, J. H., BEERMAN, H., INGRAHAM, N. R. JR., LENTZ, J. W., MORGAN, H. G., STEELE, W., AND ROSE, E. K. Penicillin in neurosyphilis. Effect on blood and spinal fluid. *J.A.M.A.*, 1945, *128:* 653–654.

GAMMON, G. D., STOKES, J. H., LENTZ, J. W., STEELE, W. H., ROSE, E. K., SCOTT, J., SCOTT, D. M. JR., AND ORNSTEEN, A. M. Immediate and early effects of penicillin on syphilis of the central nervous system. *Tr. Am. Neurol. A.*, 1944, pp. 65–69.

KLAUDER, J. V., AND DUBLIN, G. J. Syphilitic uveitis; diagnosis, Herxheimer reaction and results of various treatments, including penicillin therapy. *Arch. Ophth.* 1946, *35:* 384–399.

LENTZ, J. W., INGRAHAM, N. R., JR., BEERMAN, H., AND STOKES, J. H. Penicillin in the prevention and treatment of congenital syphilis. Report on experience with the treatment of fourteen pregnant women with early syphilis and nine infants with congenital syphilis. *J.A.M.A.*, 1944, *126:* 408–413.

MAGNUSON, H. J., AND EAGLE, H. The retardation and suppression of experimental early syphilis by small doses of penicillin comparable to those used in the treatment of gonorrhea. *Am. J. Syph., Gonor., & Ven. Dis.*, 1945, *29:* 587–596.

MOORE, J. E., MAHONEY, J. F., SCHWARTZ, W., STERNBERG, T., AND WOOD, W. B. The treatment of early syphilis with penicillin; a preliminary report of 1,418 cases. *J.A.M.A.,* 1944, *126:* 67–73.

NELSON, R. A., AND DUNCAN, L. Acute syphilitic meningitis treated with penicillin. *Bull. Johns Hopkins Hosp.,* 1944, *75:* 327–352. *Am. J. Syph., Gonor., & Ven. Dis.,* 1945, *29:* 141–164.

NELSON, R. A., AND DUNCAN, L. Penicillin in the treatment of early syphilis resistant to arsenic and bismuth. *Am. J. Syph., Gonor., & Ven. Dis.,* 1945, *29:* 1–18.

NEURATH, H., VOLKIN, E., ERICKSON, J. O., PUTNAM, F. W., CRAIG, H. W., COOPER, G. R., SHARP, D. G., TAYLOR, A. R., AND BEARD, J. W. The serological diagnosis of syphilis. *Science,* 1945, *101:* 68–69.

MCDERMOTT, W., AND NELSON, R. A. The transfer of penicillin into the cerebrospinal fluid following parenteral administration. *Am. J. Syph., Gonor., & Ven. Dis.,* 1945, *29:* 403–415.

MCDERMOTT, W., BENOIT, M., AND DUBOIS, R. Time-dose relationships of penicillin therapy. III. Regimens used in early syphilis. *Am. J. Syph., Gonor., & Ven. Dis.,* 1945, *29:* 345–352.

PLATOU, R. V., HILL, A. J., INGRAHAM, N. R., GOODWIN, M. S., WILKINSON, E. E., AND HANSEN, A. E. Penicillin in the treatment of infantile congenital syphilis. A brief preliminary note. *J.A.M.A.,* 1945, *127:* 582.

SCHOCH, A. G., AND ALEXANDER, L. J. Infectious and serologic relapse during intensive arsenotherapy of early syphilis. *Am. J. Syph., Gonor., & Ven. Dis.,* 1944, *28:* 221–227.

STOKES, J. H., BEERMAN, H., INGRAHAM, N. R., JR., LENTZ, J. W., MORGAN, H. G., STEELE, W., ROSE, E. K., AND GYORGY, P. Penicillin in late syphilis; An interim report. *Am. J. Syph., Gonor., & Ven. Dis.,* 1945, *29:* 313–333.

STOKES, J. H., STERNBERG, T. H., SCHWARTZ, W. H., MAHONEY, J. F., MOORE, J. E., AND WOOD, W. BARRY. The action of penicillin in late syphilis, including neurosyphilis, benign late syphilis, and late congenital syphilis; preliminary report. *J.A.M.A.,* 1944, *126:* 73–79.

TUCKER, H. A., Penicillin treatment of Erb's syphilitic spinal spastic paraplegia. Report of four cases, one with autopsy. *Bull. Johns Hopkins Hosp.,* 1946, *78:* 161–179.

VOLKIN, E., ERICKSON, J. O., CRAIG, H. W., AND NEURATH, H. A note on the effect of choline chloride on the Kahn flocculation reaction. *J. Am. Chem. Soc.,* 1945, *67:* 2210–2212.

WILKINSON, E. E., SAUNDERS, W. H., AND HANSEN, A. E. Penicillin in the treatment of congenital syphilis. *Texas State J. Med.,* 1945, *41:* 401–404.

Tropical Diseases

ADDIS, C. J. Phlebotomus (Dampfomyia) anthophorus, n. sp., and Phlebotomus diabolicus Hall from Texas (Diptera: Psychodidae). *J. Parasitol.,* 1945, *31:* 119–127.

CHANG, S. L. "Destruction of micro-organisms." *J. Am. Water Works A.,* 1944, *36:* 1192–1207.

CHANG, SHIH LU. Applicability of oxidation potential measurements in determining the concentration of germicidally active chlorine in water. *J. New England Water Works A.*, 1945, *59:* 79–101.

CHANG, SHIH LU. Studies on Endamoeba histolytica. III. Destruction of cysts of Endamoeba histolytica by a hypochlorite solution, chloramines in tap water and gaseous chlorine in tap water of varying degrees of pollution. *War Medicine*, 1944, *5:* 46–55.

CULBERTSON, J. T., ROSE, H. M., AND OLIVER-GONZALEZ, J. Chemotherapy of filariasis due to Wuchereria bancrofti with neostibosan. *Am. J. Hyg.*, 1946, *43:* 145–151.

CULBERTSON, J. T., ROSE, H. M., AND OLIVER-GONZALEZ, J. The chemotherapy of human filariasis by the administration of neostibosan. *Am. J. Trop. Med.*, 1945, *25:* 403–406.

FAIR, G. M., CHANG, SHIH LU, TAYLOR, M. P., AND WINEMAN, M. A. Destruction of water-borne cysts of Entamoeba histolytica by synthetic detergents. *Am. J. Public Health*, 1945, *35:* 228–232.

GELLHORN, A., AND VAN DYKE, H. B. The tissue distribution and the excretion of antimony after administration of tervalent and quinquevalent antimonials. *Federation Proc.*, 1946, *5:* 178–179.

GELLHORN, A., VAN DYKE, H. B., PYLES, W. J., AND TUPIKOVA, N. A. Amyloidosis in hamsters with leishmaniasis. *Proc. Soc. Exper. Biol. & Med.*, 1946, *61:* 25–30.

VAN DYKE, H. B., AND GELLHORN, A. Chemotherapeutic studies in experimental leishmaniasis. *Federation Proc.*, 1946, *5:* 209.

VAN DYKE, H. B., AND GELLHORN, A. Chemotherapy of experimental leishmaniasis. *Proc. Soc. Exper. Biol. & Med.*, 1946, *61:* 403–405.

Mycotic Infections

AJELLO, L., KEENEY, E. L., AND BROYLES, E. N. Observations on the incidence of Tinea pedis in a group of men entering military life. *Bull. Johns Hopkins Hosp.*, 1945, *77:* 440–447.

HILLEGAS, A. B., AND CAMP, E. The testing of fungicides insoluble in water. *J. Invest. Dermat.*, 1945, *6:* 217–226.

HOPKINS, J. G., HILLEGAS, A. B., CAMP, E., LEDIN, R. B., AND REBELL, G. Treatment and prevention of dermatophytosis and related conditions. *Bull. U. S. Army Med. Dept.*, 1944, June: 42–53.

KEENEY, E. L. The fungistatic and fungicidal effect of sodium propionate on common pathogens. *Bull. Johns Hopkins Hosp.*, 1943, *73:* 379–390.

KEENEY, E. L. Medical mycology. *M. Clin. North America*, 1945, March: 323–342.

KEENEY, E. L. A protective cabinet for investigators studying Coccidioides immitis and other infectious fungi. *Bull. Johns Hopkins Hosp.*, 1946, *78:* 113–118.

KEENEY, E. L., AJELLO, L., BROYLES, E. N., AND LANKFORD, E. Propionate and undecylenate ointments in the treatment of Tinea pedis and an in vitro comparison of their fungistatic and antibacterial effects with other ointments. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 417–439.

KEENEY, E. L., AJELLO, L., AND LANKFORD, E. Studies on common pathogenic fungi and on Actinomyces bovis. I. In vitro effect of fatty acids. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 377–392.

KEENEY, E. L., AJELLO, L., AND LANKFORD, E. Studies on common pathogenic fungi

and on Actinomyces bovis. II. In vitro effect of sulfonamides. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 393–409.

KEENEY, E. L., AJELLO, L., AND LANKFORD, E. Studies on common pathogenic fungi and on Actinomyces bovis. III. In vitro effect of penicillin. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 410–416.

KEENEY, E. L., AJELLO, L., LANKFORD, E., AND MARY, L. Sodium caprylate. A new and effective treatment for dermatomycosis of the feet. *Bull. Johns Hopkins Hosp.*, 1945, *77:* 422–436.

KEENEY, E. L., AND BROYLES, E. N. Sodium propionate in the treatment of superficial fungous infections. *Bull. Johns Hopkins Hosp.*, 1943, *73:* 479–487.

KEENEY, E. L., LANKFORD, E., AND AJELLO, L. The bacteriostatic and bactericidal effects of fatty acid salts on bacteria in broth cultures. *Bull. Johns Hopkins Hosp.*, 1945, *77:* 437–439.

LEISE, J. M., AND JAMES, L. H. Relationship between pathogenicity and pH tolerance of microorganisms. *Science,* 1945, *101:* 437–438.

LEISE, J. M., AND JAMES, L. H. An alkaline medium and procedure for the selection of dermatophytes in the presence of saprophytic fungi. *J. Lab. & Clin. Med.,* 1945, *30:* 119–131.

LEISE, J. M., AND JAMES, L. H. The isolation of dermatophytes: A new procedure for use in the presence of saprophytic fungi, especially in mixed cultures and from leather. *Arch. Dermat. & Syph.,* 1946, *53:* 481–495.

Convalescence

(see also SURGERY, Wounds and Burns, for convalescence from burns.)

ALBANESE, A. A. Studies on human blood proteins. I. The isoleucine deficiency of hemoglobin. *J. Biol. Chem.,* 1945, *157:* 613–619.

ALBANESE, A. A. The utilization of d-amino acids by man. II. Cystine. *J. Biol. Chem.,* 1945, *158:* 101–105.

ALBANESE, A. A. Colorimetric estimation of phenylalanine in some biological products. *J. Biol. Chem.,* 1944, *155:* 291–298.

ALBANESE, A. A. Amino acid analysis of some common vegetables. Method for carbohydrate-free extraction of nitrogen from fresh vegetables. *Indust. & Engin. Chem.,* 1944, *16:* 609–611.

ALBANESE, A. A. The utilization of d-amino acids by man. I. Tryptophane, methionine, and phenylalanine. *Bull. Johns Hopkins Hosp.,* 1944, *75:* 175–183.

ALBANESE, A. A., AND FRANKSTON, J. E. The colorimetric determination of arginine in protein hydrolysates and human urine. *J. Biol. Chem.,* 1945, *159:* 185–194.

ALBANESE, A. A., AND FRANKSTON, J. E. The estimation of tryptophane in human urine. *J. Biol. Chem.,* 1945, *157:* 59–68.

ALBANESE, A. A., AND FRANKSTON, J. E. A difference in the metabolism of l- and dl-tryptophane in the human. *J. Biol. Chem.,* 1944, *155:* 101–108.

ALBANESE, A. A., AND FRANKSTON, J. E. The dietary role of histidine in the immature and adult rat. *Bull. Johns Hopkins Hosp.,* 1945, *77:* 61–67.

ALBANESE, A. A., FRANKSTON, J. E., AND IRBY, V. The estimation of methionine in protein hydrolysates and human urine. *J. Biol. Chem.,* 1944, *156:* 293–302.

ALBANESE, A. A., FRANKSTON, J. E., AND IRBY, V. The utilization of d-amino acids by man. IV. Acetyltryptophane. *J. Biol. Chem.,* 1945, *160:* 31–34.

ALBANESE, A. A., FRANKSTON, J. E., AND IRBY, V. The utilization of d-amino acids by man. V. Histidine. *J. Biol. Chem.,* 1945, *160:* 441–447.

ALBANESE, A. A., AND HOLT, L. E., JR. Observations on a diet deficient in both methionine and cystine in man. *Bull. Johns Hopkins Hosp.,* 1944, *74:* 308–312.

ALBANESE, A. A., HOLT, L. E., JR., FRANKSTON, J. E., AND IRBY, V. Observations on a histidine deficient diet in man. *Bull. Johns Hopkins Hosp.,* 1944, *74:* 251–258.

ALBANESE, A. A., AND IRBY, V. Determination of urinary amino nitrogen by the copper method. *J. Biol. Chem.,* 1944, *153:* 583–588.

ALBANESE, A. A., IRBY, V., AND FRANKSTON, J. E. The utilization of d-amino acids by man. III. Arginine. *J. Biol. Chem.,* 1945, *160:* 25–30.

ALBANESE, A. A., AND WAGNER, D. L. Method for colorimetric determination of potassium in biologic products. *J. Lab. & Clin. Med.,* 1945, *30:* 280–284.

ALBANESE, A. A., AND WANGERIN, D. M. The creatine and creatinine excretion of normal adult males. *Science,* 1944, *100:* 58–60.

ARMSTRONG, W. D., KNOWLTON, M., AND GOUZE, M. Influence of estradiol and testosterone propionates on skeletal atrophy from disuse and on normal bones of mature rats. *Endocrinology,* 1945, *36:* 313–322.

BRODMAN, K., MITTLEMANN, B., AND WOLFF, H. G. Psychologic aspects of convalescence: XX. *J.A.M.A.,* 1945, *129:* 179–187.

CARTWRIGHT, G. E., LAURITSEN, M. A., HUMPHREYS, S., JONES, P. J., MERRILL, I. M., AND WINTROBE, M. M. The anemia associated with chronic infection. *Science,* 1946, *103:* 72–73.

Co TUI. The value of protein and its chemical components (amino acids) in surgical repair. *Bull. New York Acad. Med.,* 1945, *21:* 631–655.

Co TUI, BARCHAM, I., MULHOLLAND, J. H., KUTISKER, M. J., AND WRIGHT, A. M. The construction and use of a bedside ergograph. *Ann. Surg.,* 1944, *120:* 123–128.

Co TUI, BARCHAM, I., AND SHAFIROFF, B. G. P. Ligation of the thoracic duct and the posthemorrhage plasma protein level. *Surg., Gynec., & Obst.,* 1944, *79:* 37–40.

Co TUI, WRIGHT, A. M., MULHOLLAND, J. H., BREED, E. S., BARCHAM, I., AND GOULD, D. Studies in surgical convalescence. II. A preliminary study of the nitrogen loss in exudates in surgical conditions. *Ann. Surg.,* 1945, *121:* 223–230.

Co TUI, WRIGHT, A. M., MULHOLLAND, J. H., CARABBA, V., BARCHAM, I., AND VINCI, V. J. Studies on surgical convalescence. I. Sources of nitrogen loss post-gastrectomy and effect of high amino-acid and high caloric intake on convalescence. *Ann. Surg.,* 1944, *120:* 99–122.

Co TUI, WRIGHT, A. M., MULHOLLAND, J. H., GALVIN, T., BARCHAM, I., AND GERST, G. R. The hyperalimentation treatment of peptic ulcer with amino acids (protein hydrolysate) and dextrimaltose. *Gastroenterology,* 1945, *5:* 5–17.

FORBES, A. P. The 17-ketosteroid excretion in stress. *Privately printed by Josiah Macy, Jr. Foundation,* 1942.

FORBES, A. P., REIFENSTEIN, E. C., JR., KINSELL, L. W., AND ALBRIGHT, F. Is testosterone therapy indicated in female patients with Addison's disease? *Endocrinology,* 1944, *35:* 224.

GROSSMAN, C. M., SAPPINGTON, T. S., BURROWS, B. A., LAVIETES, P. H., AND PETERS,

J. P. Nitrogen metabolism in acute infections. *J. Clin. Investigation*, 1945, *24:* 523-531.

HOLT, L. E. JR., AND ALBANESE, A. A. Observations on amino acid deficiencies in man. *Tr. A. Am. Physicians*, 1944, *58:* 143-156.

HOLT, L. E., JR., ALBANESE, A. A., FRANKSTON, J. E., AND IRBY, V. The tryptophane requirement of man as determined by nitrogen balance and by excretion of tryptophane in urine. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 353-358.

HOWARD, J. E. Protein metabolism during convalescence after trauma: Recent studies. *Arch. Surg.*, 1945, *50:* 166-170.

HOWARD, J. E. Metabolic observations on patients convalescent from fracture. *Tr. A. Am. Physicians*, 1944, *58:* 162-170.

HOWARD, J. E., BIGHAM, R. S. JR., EISENBERG, H., WAGNER, D., AND BAILEY, E. Studies on convalescence. IV. Nitrogen and mineral balances during starvation and graduated feeding in healthy young males at bed rest. *Bull. Johns Hopkins Hosp.*, 1946, *78:* 282-307.

HOWARD, J. E., PARSON, W., AND BIGHAM, R. S. JR. Studies on patients convalescent from fracture. III. The urinary excretion of calcium and phosphorus. *Bull. Johns Hopkins Hosp.*, 1945, *77:* 291-313.

HOWARD, J. E., PARSON, W., STEIN, K. E., EISENBERG, H., AND REIDT, V. Studies on fracture convalescence. I. Nitrogen metabolism after fracture and skeletal operations in healthy males. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 156-168.

HOWARD, J. E., WINTERNITZ, J., PARSON, W., BIGHAM, R. S. JR., AND EISENBERG, H. Studies on fracture convalescence. II. The influence of diet on post-traumatic nitrogen deficit exhibited by fracture patients. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 209-224.

LUND, C. C., AND LEVENSON, S. M. Protein in surgery. *J.A.M.A.*, 1945, *128:* 95-100.

REIFENSTEIN, E. C. JR. Protein anabolic activity of steroid compounds. *Privately printed by Josiah Macy, Jr. Foundation.*

REIFENSTEIN, E. C. JR., FORBES, A. P., ALBRIGHT, F., DONALDSON, E., AND CARROLL, E. Effect of methyl testosterone on urinary 17-ketosteroids of adrenal origin. *J. Clin. Investigation*, 1945, *24:* 416-434.

REIFENSTEIN, E. C. JR., FORBES, A. P., KINSELL, L. W., AND ALBRIGHT, F. The use of the somatotropic properties of testosterone preparation in chronic debility. *Endocrinology*, 1944, *35:* 213.

RIEGEL, C., RHOADS, J. E., KOOP, C. E., GRIGGER, R. P., BULLITT, L., AND BARRUS, D. Dietary requirements for nitrogen equilibrium in the period immediately following certain major surgical operations. *Am. J. M. Sc.*, 1945, *210:* 133-134.

STARR, I., AND MAYOCK, R. L. Convalescence from surgical procedures. I. Studies of the circulation lying and standing, of tremor, and of a program of bed exercises and early rising. *Am. J. M. Sc.*, 1945, *210:* 701-713.

STARR, I., MAYOCK, R. L., AND BATTLES, M. G. Convalescence from surgical procedures. II. Studies of various physiological responses to a mild exercise test. *Am. J. M. Sc.*, 1945, *210:* 713-725.

STARR, I., MAYOCK, R., AND BATTLES, M. G. Experience with mild exercise as a test for the convalescent state. *Am. J. M. Sc.*, 1945, *210:* 133.

TAYLOR, H. L., ERICKSON, L., HENSCHEL, A., AND KEYS, A. The effect of bed rest on the blood volume of normal young men. *Am. J. Physiol.*, 1945, *144:* 227-232.

Analgesics

JONES, C. M., AND CHAPMAN, W. P. Comparative study of analgesic effect of morphine sulfate and monoacetylmorphine. *Arch. Int. Med.*, 1944, *73:* 322–328.

Cardiovascular Defects

BRANNON, E. S., WEENS, H. S., AND WARREN, J. V. Atrial septal defect: Study of hemodynamics by the technique of right heart catheterization. *Am. J. M. Sc.*, 1945, *210:* 480–491.

CRESCITELLI, F., AND GARDNER, E. The application of a hot wire and thermocouple for recording surface pulsations in the human body. *J. Lab. & Clin. Med.*, 1945, *30:* 63–74.

FENN, G. K., KERR, W. J., LEVY, R. L., STROUD, W. D., AND WHITE, P. D. Re-examination of 4,994 men rejected for general military service because of the diagnosis of cardiovascular defects. Individual reports by the chairmen of special medical advisory boards in five cities in which the combined study was made. *Am. Heart J.*, 1944, *27:* 435–501.

HILLMAN, C. C., LEVY, R. L., STROUD, W. D., AND WHITE, P. D. Studies of blood pressure in Army officers; observations based on an analysis of the medical records of 22,741 officers of the United States Army. *J.A.M.A.*, 1944, *125:* 699–701.

LEVY, R. L., HILLMAN, C. C., STROUD, W. D., AND WHITE, P. D. Transient hypertension: Its significance in terms of later development of sustained hypertension and cardiovascular-renal disease. *J.A.M.A.*, 1944, *126:* 829–833.

LEVY, R. L., STROUD, W. D., AND WHITE, P. D. Report of reexamination of 4,994 men disqualified for general military service, because of the diagnosis of cardiovascular defects. A combined study made by special medical advisory boards in Boston, Chicago, New York, Philadelphia, and San Francisco. *J.A.M.A.*, 1943, *123:* 937–944 and 1029–1035.

LEVY, R. L., WHITE, P. D., STROUD, W. D., AND HILLMAN, C. C. Transient hypertension. The relative prognostic importance of various systolic and diastolic levels. *J.A.M.A.*, 1945, *128:* 1059–1061.

LEVY, R. L., WHITE, P. D., STROUD, W. D., AND HILLMAN, C. C. Transient tachycardia. Prognostic significance alone and in association with transient hypertension. *J.A.M.A.*, 1945, *129:* 585–588.

Neuropsychiatry

(See also SURGERY, Neurology and Neurosurgery for psychiatric and psychologic aspects of head injury.)

ADLER, A. Neuropsychiatric complications in victims of Boston's Cocoanut Grove Disaster. *J.A.M.A.*, 1943, *123:* 1098–1101.

BILLINGS, E. G., EBAUGH, F. G., MORGAN, D. W., O'KELLY, L. I., SHORT, G. B., AND GOLDING, F. C. Comparison of one hundred Army psychiatric patients and one hundred enlisted men. *War Medicine*, 1943, *4:* 283–298.

COHEN, M. E., JOHNSON, R. E., COBB, S., CHAPMAN, U. P., AND WHITE, P. D. Studies of work and discomfort in patients with neurocirculatory asthenia. *J. Clin. Investigation*, 1944, *23:* 934.

DIETHELM, O. Value of brief psychotherapeutic interviews in a rehabilitation service. *Tr. Am. Neurol. A.,* 1944, *70:* 30–32.

HARRIS, R. E., AND CHRISTIANSEN, C. Prediction of response to brief psychotherapy. *J. Psychol.,* 1946, *21:* 269–284.

LEVINE, K. N. A comparison of Graphic Rorschach productions with scoring categories of the verbal Rorschach record in normal states, organic brain disease, neurotic and psychotic disorders. *Arch. Psychol.,* 1943, *40:* 5–63.

MITTELMANN, B., WEIDER, A., BRODMAN, K., WECHSLER, D., AND WOLFF, H. G. Personality and psychosomatic disturbances in patients on medical and surgical wards: A survey of 450 admissions. *Psychosomatic Medicine,* 1945, *7:* 220–223.

RENNIE, T. A. C. Psychiatric rehabilitation therapy. *Am. J. Psychiat.,* 1945, *101:* 476–485.

RUESCH, J. Chronic disease and psychological invalidism — a psychosomatic study. *Psychosomatic Medicine Monograph 9,* 1946 (Am. Soc. Research in Psychosomatic Problems, New York).

RUESCH, J. Personality structure, lactic acid production, and work performance in psychiatric patients. *J. Psychol.,* 1945, *20:* 381–390.

WEIDER, A., BRODMAN, K., MITTELMANN, B., WECHSLER, D., AND WOLFF, H. G. Cornell Service Index: A method for quickly assaying personality and psychosomatic disturbances in men in the armed forces. *War Medicine,* 1945, *7:* 209–213.

WEIDER, A., MITTELMANN, B., WECHSLER, D., AND WOLFF, H. G. The Cornell Selectee Index: A method for quick testing of selectees for the armed forces. *J.A.M.A.,* 1944, *124:* 224–228.

WEIDER, A., WECHSLER, D., MITTELMANN, B., AND WOLFF, H. G. The Cornell Selectee Index: Short form to be used at induction, at reception and during hospitalization. *Privately printed by Josiah Macy, Jr. Foundation,* 1944.

WHITE, P. D., COBB, S., CHAPMAN, W. P., COHEN, M. E., AND BADAL, D. W. Observations on neurocirculatory asthenia. *Tr. A. Am. Physicians,* 1944, *58:* 129–136.

SURGERY

Wounds and Burns

ABBOTT, W. E., HIRSHFELD, J. W., AND MEYER, F. L. Metabolic alterations following thermal burns. II. Changes in the plasma volume and plasma protein in the convalescent phase. *Surg., Gynec., & Obst.,* 1945, *81:* 25–30.

ABBOTT, W. E., MEYER, F. L., HIRSHFELD, J. W., AND GRIFFIN, G. E. Metabolic alterations following thermal burns. IV. The effect of treatment with whole blood and electrolyte solution or with plasma following an experimental burn. *Surgery,* 1945, *17:* 794–804.

ABBOTT, W. E., PILLING, M. A., GRIFFIN, G. E., HIRSHFELD, J. W., AND MEYER, F. L. Metabolic alterations following thermal burns. V. The use of whole blood and an electrolyte solution in the treatment of burned patients. *Ann. Surg.,* 1945, *122:* 678–692.

ALTEMEIER, W. A. Inactivation of penicillin by various gram-negative bacteria. *Surg., Gynec., & Obst.,* 1945, *81:* 379–383.

ALTEMEIER, W. A. Penicillin in surgery. *South. M. J.,* 1944, *37:* 494–506. *Cincinnati J. Med.,* 1944, *25:* 340–360. *J.-Lancet,* 1945, *65:* 93–100.

ALTEMEIER, W. A. Treatment of acute hematogenous osteomyelitis with penicillin. *Ohio State M. J.,* 1946, *42:* 489–496.

ALTEMEIER, W. A. AND HELMSWORTH, J. A. Penicillin therapy in acute osteomyelitis. *Surg., Gynec., & Obst.,* 1945, *81:* 138–157.

ANDERSON, D. G., HOWARD, L. G., AND RAMMELKAMP, C. H. Penicillin in the treatment of chronic osteomyelitis. A report of forty cases. *Arch. Surg.,* 1944, *49:* 245–257.

BAKER, R. D. The internal lesions in burns with special reference to the liver and to splenic nodules. An analysis of 96 autopsies. *Am. J. Path.,* 1945, *21:* 717–739.

BAKER, R. D. Untoward effects of various substances recommended for burns or wounds. Experimental tests on rats. *Arch. Surg.,* 1944, *48:* 300–304.

BAKER, R. D. AND HANDLER, P. Animal experiments with tannic acid suggested by the tannic acid treatment of burns. *Ann. Surg.,* 1943, *118:* 417–426.

BLAIR, J. E., CARR, M., AND BUCHMAN, J. The action of penicillin on staphylococci. *J. Immunol.,* 1946, *52:* 281–292.

BRUNEAU, J. AND HEINBECKER, P. Effects of cooling on experimentally infected tissues. *Ann. Surg.,* 1944, *120:* 716–726.

BUCHMAN, J. AND BLAIR, J. E. Penicillin in the treatment of chronic osteomyelitis. A preliminary report. *Arch. Surg.,* 1945, *51:* 81–92.

BUCHMAN, J. AND BLAIR, J. E. Report on the use of penicillin in the treatment of staphylococcal infections with special reference to acute and chronic osteomyelitis and several collateral studies. *Bull. Hosp. Joint Diseases,* 1945, *6:* 114–125.

CANNON, B. AND COPE, O. Rate of epithelial regeneration. A clinical method of measurement, and the effect of various agents recommended in the treatment of burns. *Ann. Surg.,* 1943, *117:* 85–92.

CLOWES, G. H. A., JR., LUND, C. C., AND LEVENSON, S. M. The surface treatment of burns. A comparison of results of tannic acid, silver nitrate, triple dye, and vaseline or boric ointment as surface treatments in 150 cases. *Ann. Surg.,* 1943, *118:* 761–779.

CONNOR, G. J. AND HARVEY, S. C. Symposium on burns. The healing of deep thermal burns. A preliminary report. *Ann. Surg.,* 1944, *120:* 362–366.

COPE, O. Care of the victims of the Cocoanut Grove fire at the Massachusetts General Hospital. *New England J. Med.,* 1943, *229:* 138–146.

COPE, O. Deep burns and penicillin. *Bull. New England Med. Center,* 1944, *6:* 255.

COPE, O. The chemical aspects of burn treatment. *J.A.M.A.,* 1944, *125:* 536–543.

COPE, O. The treatment of the surface burns. *Ann. Surg.,* 1943, *117:* 885–893.

COPE, O. AND MOORE, F. D. A study of capillary permeability in experimental burns and burn shock using radioactive dyes in blood and lymph. *J. Clin. Investigation,* 1944, *23:* 241–257.

COPE, O., NATHANSON, I. T., ROURKE, G. M., AND WILSON, H. Metabolic observations. *Ann. Surg.,* 1943, *117:* 937–958.

CO TUI, WRIGHT, A. M., MULHOLLAND, J. H., BARCHAM, I., AND BREED, E. S. The nutritional care of cases of extensive burns, with special reference to the oral use of amino-acids (amigen) in three cases. *Ann. Surg.,* 1944, *119:* 815–823.

EVANS, E. I. AND HOOVER, M. J. The sulfanilamide ointment treatment of severe burns. *Surg., Gynec., & Obst.,* 1943, *77:* 367–375.

EVANS, E. I., HOOVER, M. J., AND JAMES, G. W. The absorption of sulfonamides from the burn surface. *Surg., Gynec., & Obst.,* 1945, *80:* 297–302.

FINLAND, M., DAVIDSON, C. S., AND LEVENSON, S. M. Chemotherapy and control of infection among victims of the Cocoanut Grove disaster. *Surg., Gynec., & Obst.,* 1946, *82:* 151–173.

FINLAND, M., RITVO, M., DAVIDSON, C. S., AND LEVENSON, S. M. Roentgenologic findings in the lungs of victims of the Cocoanut Grove disaster. *Am. J. Roentgenol.,* 1946, *55:* 1–15.

FORBES, J. C. AND EVANS, E. I. Tannic acid and liver necrosis. *Surg., Gynec., & Obst.,* 1943, *76:* 612–613.

FOX, C. L., JR. Certain new considerations in local sulfonamide therapy. *Bull. New York Acad. Med.,* 1943, *19:* 661–662.

GOODPASTOR, W. E., LEVENSON, S. M., TAGNON, H. J., LUND, C. C., AND TAYLOR, F. H. L. A clinical and pathologic study of the kidney in patients with thermal burns. *Surg., Gynec., & Obst.,* 1946, *82:* 652–670.

GREEN, R. W., LEVENSON, S. M., AND LUND, C. C. Nylon backing for dermatome grafts. *New England J. Med.,* 1945, *233:* 268–270.

HALL, I. C. A war-time study of civilian wounds. *Proc. New York State A. Pub. Health Libraries,* 1944, *24:* 16.

HALL, I. C. The occurrence and possible significance of Bacillus tetani in compound fractures, lacerations, gunshot wounds, and burns. *Surgery,* 1945, *18:* 377–388.

HALL, I. C. The occurrence of Bacillus botulinus, types A and B, in accidental wounds. *J. Bact.,* 1945, *50:* 213–217.

HALL, I. C. The occurrence of Bacillus histolyticus in accidental wounds without recognized specific infection. *Surgery,* 1945, *18:* 369–377.

HANDLER, P. AND BAKER, R. D. The toxicity of orally administered tannic acid. *Science,* 1944, *99:* 393.

HARTMAN, F. W. Curling's ulcer in experimental burns. *Ann. Surg.,* 1945, *121:* 54–64.

HARTMAN, F. W., AND ROMENCE, H. L. Liver necrosis in burns. *Ann. Surg.,* 1943, *118:* 402–416.

HAWN, C. V. Z., BERING, E. A., JR., BAILEY, O. T., AND ARMSTRONG, S. H., JR. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XIX. A note on the use of fibrinogen and thrombin in the surface treatment of burns. *J. Clin. Investigation,* 1944, *23:* 580–585.

HIRSHFELD, J. W., ABBOTT, W. E., PILLING, M. A., HELLER, C. G., MEYER, F., WILLIAMS, H. H., RICHARDS, A. J., AND OBI, R. Metabolic alterations following thermal burns. III. Effect of variations in food intake on nitrogen balance of burned patients. *Arch. Surg.,* 1945, *50:* 194–200.

HIRSHFELD, J. W., PILLING, M. A., BUGGS, C. W., AND ABBOTT, W. E. Penicillin and skin grafting. *J.A.M.A.,* 1944, *125:* 1017–1019.

HIRSHFELD, J. W., WILLIAMS, H. H., ABBOTT, W. E., HELLER, C. G., AND PILLING, M. A. Significance of the nitrogen loss in the exudate from surface burns. *Surgery,* 1944, *15:* 766–773.

HOGE, W. Presence of Clostridia in burns with reference to tetanus. *Quart. Bull. Northwestern Univ. Med. Sch.*, 1945, *19:* 111–112.

HOLMES, L. F. AND WILSON, M. E. A micrococcus producing a mulberry-colored pigment. *J. Bact.*, 1945, *49:* 311–312.

HOLMES, L. F. AND WILSON, M. E. Gentian violet-blood agar plates used in aerobic and anaerobic cultures of wounds. *J. Lab. & Clin. Med.*, 1944, *29:* 1090–1093.

HOWES, E. L. Local chemotherapy of wounds. Tissue toxicity of certain antibacterial substances. *Surg., Gynec. & Obst.*, 1946, *83:* 1–14.

JENKINS, H. P., ALLEN, J. G., OWENS, F. M., JR., SCHAFER, P. W., AND DRAGSTEDT, L. R. Further studies on the preparation and use of sulfathiazole ointment in the treatment of burns. *Surg., Gynec. & Obst.*, 1945, *80:* 85–96.

LAM, C. R. Recent advances in the treatment of burns. *Indust. Med.*, 1945, *14:* 610.

LAM, C. R. The general care of the burned patient. *J.A.M.A.*, 1944, *125:* 543–546.

LAM, C. R. AND MCCLURE, R. D. Penicillin as an adjunct in the skin grafting of severe burns. *Proc. Am. Federation Clin. Research*, 1944, *1:* 56.

LAM, C. R. AND PUPPENDAHL, M. The pyruvic acid method of burn slough removal. An experimental investigation. *Ann. Surg.*, 1945, *121:* 866–871.

LARGE, A. AND HEINBECKER, P. Refrigeration in clinical surgery. *Ann. Surg.*, 1944, *120:* 707–715.

LEE, W. E. AND RHOADS, J. E. The present status of the tannic acid method in the treatment of burns. *J.A.M.A.*, 1944, *125:* 610–612.

LEE, W. E., WALKER, J., JR., PRESSLEY, L., AND RHOADS, J. E. Certain recent advances in burn therapy. *Pennsylvania M. J.*, 1945, *48:* 563.

LEOPOLD, I. H. Intravitreal penetration of penicillin and penicillin therapy of infections in the vitreous. *Arch. Ophth.*, 1945, *33:* 211–216.

LEOPOLD, I. H. Local toxic effect of detergents on ocular structures. *Arch. Ophth.*, 1945, *34:* 99–102.

LEOPOLD, I. H., HOLMES, L. F., AND LAMOTTE, W. O., JR. Local versus systemic penicillin therapy of rabbit corneal ulcer produced by gram-negative rod. *Arch. Ophth.*, 1944, *32:* 193–195.

LEOPOLD, I. H. AND LAMOTTE, W. O., JR. Penetration of penicillin in rabbit eyes with normal, inflamed, and abraded corneas. *Arch. Ophth.*, 1945, *33:* 43–46.

LEOPOLD, I. H. AND NICHOLS, A. Intraocular penetration of streptomycin following systemic and local administration. *Arch. Ophth.*, 1946, *35:* 33–38.

LEOPOLD, I. H. AND STEELE, W. H. Influence of local application of sulfonamide compounds and their vehicles on regeneration of corneal epithelium. *Arch. Ophth.*, 1945, *33:* 463–467.

LEVENSON, S. M., DAVIDSON, C. S., LUND, C. C., AND TAYLOR, F. H. L. The nutrition of patients with thermal burns. *Surg., Gynec. & Obst.*, 1945, *80:* 449–469.

LEVENSON, S. M. AND LUND, C. C. Dermatome skin grafts for burns in patients prepared with dry dressings and with and without penicillin. *New England J. Med.*, 1945, *233:* 607–612.

LEVENSON, S. M. AND LUND, C. C. The treatment of burns of the extremities with close fitting plaster of paris casts. *J.A.M.A.*, 1943, *123:* 272–277.

LOCKWOOD, J. S., WHITE, W. L., AND MURPHY, F. D. The use of penicillin in surgical infections. *Ann. Surg.*, 1944, *120:* 311–349.

LUND, C. C. Burns. *Rhode Island M. J.*, 1943, *26:* 197–200, 204.

Lund, C. C. The treatment of thermal burns. II. Recent developments. *New England J. Med.*, 1943, 229: 868–873.

Lund, C. C. and Browder, N. C. The estimation of areas of burns. *Surg., Gynec., & Obst.*, 1944, 79: 352–358.

Lund, C. C., Green, R., and Levenson, S. M. First aid treatment of burns. *Hospital Corps Quarterly*, 1945, Nov.: 37–39.

Lund, C. C., Green, R. W., and Levenson, S. M. Les brulures. *A pamphlet published in French by the Belgian American Educational Foundation* (New York), 1945, Dec.

Lund, C. C., Green, R. W., Taylor, F. H. L., and Levenson, S. M. Burns; collective review. *Surg., Gynec., & Obst.*, 1946, 82: 443–478.

Lyons, C. Problems of infection and chemotherapy. *Ann. Surg.*, 1943, 117: 894–902.

MacDonald, A. H., Levenson, S. M., Davidson, C. S., Tagnon, H. J., and Taylor, F. H. L. Studies on the peripheral blood in patients with thermal burns. I. Thrombocytopenia. *Science*, 1944, 99: 519.

McCarthy, M. D. A standardized back burn procedure for the white rat suitable for the study of the effects of therapeutic and toxic agents on long-term survival. *J. Lab. & Clin. Med.*, 1945, 30: 1027–1033.

McClure, R. D. and Lam, C. R. A statistical study of minor industrial burns. *J.A.M.A.*, 1943, 122: 909–911.

McClure, R. D., Lam, C. R., and Romence, H. Tannic acid and the treatment of burns; an obsequy. *Ann. Surg.*, 1944, 120: 387–398.

Meleney, F. L. A statistical analysis of a study of the prevention of infection in soft part wounds, compound fractures, and burns with special reference to the sulfonamides. *Surg., Gynec. & Obst.*, 1945, 80: 263–296.

Meleney, F. L. Chemotherapy and antibiotic therapy in surgical infections. Loose-leaf *Surgery*, Thomas Nelson & Sons, New York City, 1945, Chap. III–A, 207P–208BB.

Meleney, F. L. Recent experiences with penicillin in the treatment of surgical infections. *Bull. New York Acad. Med.*, 1944, 20: 517–537.

Meleney, F. L. Some practical points in the administration of penicillin in the treatment of surgical infections. *New York Med.*, 1945, 1: 11.

Meleney, F. L. The difficulty of evaluating drug treatment in surgical infections. *J.A.M.A.*, 1944, 124: 1021–1026.

Meleney, F. L. The study of the prevention of infection in contaminated accidental wounds, compound fractures and burns. *Ann. Surg.*, 1943, 118: 171–186.

Meleney, F. L., Friedman, S. T., and Harvey, H. D. The treatment of progressive bacterial synergistic gangrene with penicillin. *Surgery*, 1945, 18: 423–435.

Meleney, F. L., Johnson, B. A., Pulaski, E. J., and Colonna, F. Treatment of mixed infections with penicillin. I. With special reference to the adjuvant action of parachlorophenol. *J.A.M.A.*, 1946, 130: 121–124.

Meyer, F. L., Abbott, W. E., and Hirshfeld, J. W. Studies on forced feeding after burns. *Federation Proc.*, 1945, 4: 159–160.

Meyer, F. L., Joseph, S., Hirshfeld, J. W., and Abbott, W. E. Metabolic alterations following thermal burns. I. Nitrogen balance in experimental burns. *J. Clin. Investigation*, 1945, 24: 579–582.

NOTH, P. H. AND HIRSHFELD, J. W. Amebic abscess of the liver with secondary infection: Local treatment with penicillin. *J.A.M.A.*, 1944, *124:* 643–646.

O'DONNELL, C. H., POSCH, J. L., AND HIRSHFELD, J. W. A study of the value of local sulfathiazole in operative wounds in the prophylaxis of infection. *Surg., Gynec. & Obst.*, 1946, *82:* 323–326.

PARKINS, W. M., WILEY, M., CHANDY, J., AND ZINTEL, H. A. Maintenance of the blood level of penicillin after intramuscular injection. *Science*, 1945, *101:* 203–205.

SALTONSTALL, H., WALKER, J., JR., RHOADS, J. E., AND LEE, W. E. The influence of local treatment of burns on liver function. *Ann. Surg.*, 1945, *121:* 291–300.

SMELSER, G. K. The influence of vehicles and form of penicillin and sulfonamides on mitosis and healing of corneal burns. *Am. J. Ophth.*, 1946, *29:* 541–551.

SMELSER, G. K. AND OZANICS, V. Effect of chemotherapeutic agents on cell division and healing of corneal burns and abrasions in the rat. *Am. J. Ophth.*, 1944, *27:* 1063–1072.

SMELSER, G. K. AND OZANICS, V. Effect of local anesthetics on cell division and migration following thermal burns of cornea. *Arch. Ophth.*, 1945, *34:* 271–277.

TAYLOR, F. H. L. The nitrogen requirement of patients with thermal burns. *J. Indust. Hyg. & Toxicol.*, 1944, *26:* 152–155.

TAYLOR, F. H. L., DAVIDSON, C. S., AND LEVENSON, S. M. The problem of nutrition in the presence of excessive nitrogen requirement in seriously ill patients with particular reference to thermal burns. *Connecticut M. J.*, 1944, *8:* 141.

TAYLOR, F. H. L., LEVENSON, S. M., AND ADAMS, M. A. Abnormal carbohydrate metabolism in human thermal burns. Preliminary observations. *New England J. Med.*, 1944, *231:* 437–445.

TAYLOR, F. H. L., LEVENSON, S. M., DAVIDSON, C. S., AND ADAMS, M. A. Abnormal nitrogen metabolism in patients with thermal burns. *New England J. Med.*, 1943, *229:* 855–859.

TAYLOR, F. H. L., LEVENSON, S. M., DAVIDSON, C. S., ADAMS, M. A., AND MAC-DONALD, H. Abnormal nitrogen metabolism in burns. *Science*, 1943, *97:* 423.

TAYLOR, F. H. L., LEVENSON, S. M., DAVIDSON, C. S., BROWDER, N. C., AND LUND, C. C. Problems of protein nutrition in burned patients. *Ann. Surg.*, 1943, *118:* 215–224.

WALKER, J., JR. Changes in the non-protein fractions of the plasma nitrogen following extensive thermal burns. *Am. J. M. Sc.*, 1945, *209:* 413–414.

WALKER, J., JR., AND SHENKIN, H. Studies on the toxemia syndrome after burns. II. Central nervous system changes as a cause of death. *Ann. Surg.*, 1945, *121:* 301–313.

WARMER, H. AND AMLUXEN, J. Comparison of in vitro and in vivo penicillin resistance of a strain of hemolytic Staphylococcus aureus. *J. Lab. & Clin. Med.*, 1945, *30:* 419–421.

WHITE, W. L., BURNETT, W. E., BAILEY, C. P., ROSEMOND, G. P., NORRIS, C. W., FAVORITE, G. O., SPAULDING, E. H., BONDI, A., JR., AND FOWLER, R. H. Use of penicillin in prevention of postoperative empyema following lung resection. Report of a controlled study. *J.A.M.A.*, 1944, *126:* 1016–1024.

WHITE, W. L., FLIPPIN, H. F., LOCKWOOD, J. S., AND MURPHY, F. D. The indications for penicillin, its dosage and administration. *Clinics*, 1944, *3:* 309–323.

WHITE, W. L., MURPHY, F. D., LOCKWOOD, J. S., AND FLIPPIN, H. F. Penicillin in the treatment of pneumococcal, meningococcal, streptococcal, and staphylococcal meningitis. *Am. J. M. Sc.,* 1945, *210:* 1–17.

ZAMECNIK, P. C., STEPHENSON, M. L., AND COPE, O. Peptidase activity of lymph and serum after burns. *J. Biol. Chem.,* 1945, *158:* 135–144.

Gas Gangrene

ADAMS, M. H. AND HENDEE, E. D. Methods for the production of the alpha and theta toxins of Clostridium welchii. *J. Immunol.,* 1945, *51:* 249–256.

BERNHEIMER, A. W. Kinetics of lysis by Clostridium septicum hemolysin. *J. Exper. Med.,* 1944, *80:* 333–339.

BERNHEIMER, A. W. Nutritional requirements and factors affecting the production of toxin of Clostridium septicum. *J. Exper. Med.,* 1944, *80:* 321–331.

BERNHEIMER, A. W. Parallelism in the lethal and hemolytic activity of the toxin of Clostridium septicum. *J. Exper. Med.,* 1944, *80:* 309–320.

BOYD, M. J., AND LOGAN, M. A. The estimation of free formaldehyde by diffusion. *J. Biol. Chem.,* 1945, *160:* 571–583.

BYERS, S. O., TYTELL, A. A., AND LOGAN, M. A. The production of high titer Clostridium perfringens hyaluronidase (spreading factor). *J. Bact.,* 1944, *47:* 456–457.

DOWDY, A. H., SEWELL, R. L., AND VINCENT, J. G. The prophylaxis and therapeusis of Clostridial infections (gas gangrene). *New York State J. Med.,* 1944, *44:* 1890–1897.

FAVATA, B. V., DOWDY, A. H., SEWELL, R. L., AND VINCENT, J. G. The pathology of experimental clostridial infections in dogs. *Surg., Gynec. & Obst.,* 1944, *79:* 660–668.

HALL, I. C. An experimental evaluation of American commercial bivalent and pentavalent gas gangrene antitoxins. *Surg., Gynec. & Obst.,* 1945, *81:* 487–499.

HALL, I. C. The ambiguity of international antitoxic units. *Science,* 1946, *103:* 423–424.

HALL, I. C. The value of antitoxin in the prevention and treatment of malignant edema and gas gangrene. A review of observations. *Ann. Surg.,* 1945, *122:* 197–209.

HARVEY, H. D. AND MELENEY, F. L. A case of infection with Clostridium sordellii and a case of gas gangrene treated by penicillin. *Surgery,* 1944, *15:* 622–627.

KEPL, M., CALDWELL, G., AND OCHSNER, A. Use of adrenal cortical hormone in Cl. welchii infections in guinea pigs. *Proc. Soc. Exper. Biol. & Med.,* 1943, *52:* 25–26.

KEPL, M. F., OCHSNER, A., AND DIXON, J. L. Two cases of Clostridium welchii infection treated with penicillin. *J.A.M.A.,* 1944, *126:* 96–98.

LOGAN, M. A., TYTELL, A. A., DANIELSON, I. S., AND GRINER, A. M. Production of Clostridium perfringens alpha toxin. *J. Immunol.,* 1945, *51:* 317–328.

PAPPENHEIMER, A. M., JR., AND SHASKAN, E. Effect of iron on carbohydrate metabolism of Clostridium welchii. *J. Biol. Chem.,* 1944, *155:* 265–275.

ZAMECNIK, P. C., FOLCH, J., AND BREWSTER, L. Protection of animals against Cl. welchii (type A) toxin by injection of certain purified lipids. *Proc. Soc. Exper. Biol. & Med.,* 1945, *60:* 33–39.

Wound Healing

Howes, E. L. Recent advances in studying the problems of healing and their effect on the treatment of wounds and burns. *New York State J. Med.,* 1944, *44:* 2006–2011.

Lam, C. R. Chlorophyll and wound healing: Clinical studies. *Univ. Hosp. Bull., Ann Arbor, Mich.,* 1945, *11:* 42.

Large, A. and Heinbecker, P. The effect of cooling on wound healing. *Ann. Surg.,* 1944, *120:* 727–741.

Marshak, A. Healing of experimental skin wounds. *Proc. Soc. Exper. Biol. & Med.,* 1945, *58:* 63–65.

Marshak, A. and Byron, R. L., Jr. A method for studying healing of bone. *J. Bone & Joint Surg.,* 1945, *27:* 95–104.

Marshak, A. and Byron, R. L., Jr. The use of regenerating liver as a method of assay. *Proc. Soc. Exper. Biol. & Med.,* 1945, *59:* 200–202.

Marshak, A. and Walker, A. C. Effect of a chromatin derivative on the healing of skin wounds. *Proc. Soc. Exper. Biol. & Med.,* 1945, *58:* 62–63.

Marshak, A. and Walker, A. C. Effect of liver fractions on mitosis in regenerating liver. *Am. J. Physiol.,* 1945, *143:* 226–234.

Marshak, A. and Walker, A. C. Mitosis in regenerating liver. *Science,* 1945, *101:* 94–95.

Marshak, A. and Walker, A. C. Transfer of P^{32} from intravenous chromatin to hepatic nuclei. *Am. J. Physiol.,* 1945, *143:* 235–237.

Menkin, V. Chemical basis of injury in inflammation. *Arch. Path.,* 1943, *36:* 269–288.

Menkin, V. On the mechanism of fever production with inflammation. *Proc. Soc. Exper. Biol. & Med.,* 1943, *54:* 184–186.

Menkin, V. Studies on the isolation of the factor responsible for tissue injury in inflammation. *Science,* 1943, *97:* 165–167.

Menkin, V. The effect of the leukocytosis-promoting factor on the growth of cells in the bone marrow. *Am. J. Path.,* 1943, *19:* 1021–1029.

Wound Ballistics

Butler, E. G., Puckett, W. O., Harvey, E. N., and McMillen, J. H. Experiments on head wounding by high velocity missiles. *J. Neurosurg.,* 1945, *2:* 358–363.

Lamport, H. The relationship between the military loss resulting from a casualty and the days lost from active duty. *Yale J. Biol. & Med.,* 1946, *18:* 567–575.

McMillen, J. H. Shock wave pressures in water produced by impact of small spheres. *Physical Review,* 1945, *68:* 198–209.

Puckett, W. O., McElroy, W. D., and Harvey, E. N. Studies of wounds of the abdomen and thorax produced by high velocity missiles. *Mil. Surgeon,* 1946, *98:* 427–439.

Neurology and Neurosurgery

Adler, A. Mental symptoms following head injury — a statistical analysis of two hundred cases. *Arch. Neurol. and Psychiat.,* 1945, *53:* 34–43.

ADLER, A. Two different types of post-traumatic neuroses. *Am. J. Psychiat.*, 1945, *102:* 237–240.

AIRD, R. B. AND STRAIT, L. Neurophysiological studies on experimental cerebral concussions. *A. Research Nerv. & Ment. Dis., Proc.*, 1945, *24:* 283–295.

BAILEY, O. T. AND INGRAHAM, F. D. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXI. The use of fibrin foam as a hemostatic agent in neurosurgery: Clinical and pathological studies. *J. Clin. Investigation,* 1944, *23:* 591–596.

BAILEY, O. T. AND INGRAHAM, F. D. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXII. Fibrin films in neurosurgery with special reference to their use in the repair of dural defects and in the prevention of meningocerebral adhesions. *J. Clin. Investigation,* 1944, *23:* 597–660.

BERRY, C. M., GRUNDFEST, H., AND HINSEY, J. C. The electrical activity of regenerating nerves in the cat. *J. Neurophysiol.,* 1944, *7:* 103–116.

BERRY, C. M., NEUMANN, C., AND HINSEY, J. C. Nerve regeneration in cats on vitamin B_1 deficient diets. *J. Neurophysiol.,* 1945, *8:* 315–322.

BOELL, E. J. Cholinesterase activity of peripheral nerves. *J. Cell. & Comp. Physiol.,* 1945, *25:* 75–84.

BRENNER, C., FRIEDMAN, A. P., MERRITT, H. H., AND DENNY-BROWN, D. E. Post-traumatic headache. *J. Neurosurg.,* 1944, *1:* 379–392.

BRUESCH, S. R. AND RICHTER, C. P. Cutaneous distribution of peripheral nerves in Rhesus monkeys as determined by the electrical skin resistance method. *Bull. Johns Hopkins Hosp.,* 1946, *78:* 235–260.

BURT, A. S. Chemical factors in nerve growth studied in tissue culture: Vitamin B_1 and the growth of spinal ganglia. *J. Cell. & Comp. Physiol.,* 1943, *21:* 145–159.

BURT, A. S. Effect of biotin on chick spinal ganglia in tissue culture. *Proc. Soc. Exper. Biol. & Med.,* 1943, *54:* 191–193.

BURT, A. S. Growth of spinal ganglia in plasma from vitamin B_1 deficient chickens. *J. Cell. & Comp. Physiol.,* 1943, *22:* 205–222.

CLARK, S. L. Blast injury. *Quarterly Bull., Northwestern Univ. Med. School,* 1944, *18:* 81–90.

CLARK, S. L. AND WARD, J. W. Electroencephalogram of different cortical regions of normal and anaesthetized cats. *J. Neurophysiol.,* 1945, *8:* 99–112.

CLARK, S. L. AND WARD, J. W. The effects of rapid compression waves on animals submerged in water. *Surg., Gynec. & Obst.,* 1943, *77:* 403–412.

COLE, E. M. Intellectual impairment in head injury. *A. Research Nerv. & Ment. Dis., Proc.,* 1945, *24:* 473–479.

DAVIS, L., PERRET, G., AND CARROLL, W. Experimental studies in peripheral nerve surgery. I. The effect of sulfonamide drugs upon experimental gunshot wounds involving peripheral nerves. *Surg., Gynec. & Obst.,* 1944, *79:* 245–255.

DAVIS, L., HILLER, F., PERRET, G., AND CARROLL, W. Effect of sulfonamides upon experimental gunshot wounds involving peripheral nerves. *Ann. Surg.,* 1944, *120:* 494–503.

DAVIS, L., HILLER, F., PERRET, G., AND CARROLL, W. Experimental studies in peripheral nerve surgery. II. The effect of sulfonamide drugs upon the regeneration of peripheral nerves. *Surg., Gynec. & Obst.,* 1944, *79:* 250–255.

DAVIS, L., PERRET, G., HILLER, F., AND CARROLL, W. Experimental studies of peripheral nerve injuries. III. A study of recovery of function following repair by end to end suture and nerve grafts. *Surg., Gynec. & Obst.*, 1945, *80:* 35–59.

DAVIS, L., PERRET, G., AND HILLER, F. Experimental studies in peripheral nerve surgery. IV. The effect of infection on regeneration and functional recovery. *Surg., Gynec. & Obst.*, 1945, *81:* 302–308.

DENNY-BROWN, D. Disability arising from closed head injury. *J.A.M.A.*, 1945, *127:* 429–436.

DENNY-BROWN, D. Intellectual deterioration resulting from head injury. *A. Research Nerv. & Ment. Dis., Proc.*, 1945, *24:* 467–472.

DENNY-BROWN, D., ADAMS, R. D., BRENNER, C., AND DOHERTY, M. M. The pathology of injury to nerve induced by cold. *J. Neuropath. & Exper. Neurol.*, 1945, *4:* 305–323.

DENNY-BROWN, D. AND BRENNER, C. Lesion in peripheral nerve resulting from compression by spring clip. *Arch. Neurol. & Psychiat.*, 1944, *52:* 1–19.

DENNY-BROWN, D. AND BRENNER, C. Paralysis of nerve induced by direct pressure and by tourniquet. *Arch. Neurol. & Psychiat.*, 1944, *51:* 1–26.

DOW, R. S., ULETT, G., AND RAAF, J. Electroencephalographic studies immediately following head injuries. *Am. J. Psychiat.*, 1944, *101:* 174–183.

DOW, R. S., ULETT, G., AND TUNTURI, A. Electroencephalographic changes following head injuries in dogs. *J. Neurophysiol.*, 1945, *8:* 161–172.

EDDS, M. V., JR. Prevention of nerve regeneration and neuroma formation by caps of synthetic resin. *J. Neurosurg.*, 1945, *2:* 507–509.

FRIEDMAN, A. P. AND BRENNER, C. Amnestic-confabulatory syndrome (Korsakoff psychosis) following head injury. *Am. J. Psychiat.*, 1945, *102:* 61–66.

FRIEDMAN, A. P. AND BRENNER, C. Post-traumatic and histamine headache. *Arch. Neurol. & Psychiat.*, 1944, *52:* 126–130.

FRIEDMAN, A. P., BRENNER, C., AND DENNY-BROWN, D. Post-traumatic vertigo and dizziness. *J. Neurosurg.*, 1945, *2:* 36–46.

FRIEDMAN, A. P. AND MERRITT, H. H. Damage to cranial nerves resulting from head injury. *Bull. Los Angeles Neurol. Soc.*, 1944, *9:* 135–139.

FRIEDMAN, A. P. AND MERRITT, H. H. The relationship of intracranial pressure and presence of blood in the cerebrospinal fluid to the occurrence of headaches in patients with injuries to the head. *J. Nerv. & Ment. Dis.*, 1945, *102:* 1–7.

GOLDFARB, A. I., TARLOV, I. M., BOJAR, S., AND WIENER, A. S. Plasma clot tensile strength measurement: Its relation to plasma fibrinogen. *J. Clin. Investigation*, 1943, *22:* 183–190.

GRENELL, R. G. AND BURR, H. S. Electrical correlates of peripheral nerve injury: A preliminary note. *Science*, 1946, *103:* 48–49.

GROAT, R. A. Blood supply of the cat's spinal cord. *Anat. Rec.*, 1945, *91:* 278.

GROAT, R. A., MAGOUN, H. W., DEY, F. L., AND WINDLE, W. F. Functional alterations in motor and supranuclear mechanisms in experimental concussion. *Am. J. Physiol.*, 1944, *141:* 117–127. *Federation Proc.*, 1944, *3:* 14–15.

GROAT, R. A. AND PEELE, T. L. Blood pressure response to acutely increased pressure upon the spinal cord. *Am. J. Physiol.*, 1945, *144:* 578–587.

GROAT, R. A. AND PEELE, T. L. Blood pressure response to changes of pressure on the spinal cord. *Federation Proc.*, 1945, *4:* 28.

GROAT, R. A., RAMBACH, W. A., JR., AND WINDLE, W. F. Concussion of the spinal cord. *Anat. Rec.,* 1944, *88:* 434.

GROAT, R. A., RAMBACH, W. A., JR., AND WINDLE, W. F. Concussion of the spinal cord. An experimental study and a critique of the use of the term. *Surg., Gynec. & Obst.,* 1945, *81:* 63–74.

GROAT, R. A., WINDLE, W. F., AND MAGOUN, H. W. Functional and structural changes in the monkey's brain during and after concussion. *J. Neurosurg.,* 1945, *2:* 26–35.

HAMMOND, W. S., NONIDEZ, J. F., AND HINSEY, J. C. Effect of various sulfonamide compounds on nerve regeneration. *Arch. Neurol. & Psychiat.,* 1943, *50:* 499–509.

INGRAHAM, F. D. AND BAILEY, O. T. The use of products prepared from human fibrinogen and human thrombin in neurosurgery; fibrin foams as hemostatic agents; fibrin films in repair of dural defects and in prevention of meningo-cerebral adhesions. *J. Neurosurg.,* 1944, *1:* 23–39.

INGRAHAM, F. D., BAILEY, O. T., AND NULSEN, F. E. Studies on fibrin foam as a hemostatic agent in neurosurgery, with special reference to its comparison with muscle. *J. Neurosurg.,* 1944, *1:* 171–181.

KOSOL, H. L. Pretraumatic personality and psychiatric sequelae of head injury. I. Categorical pretraumatic personality status correlated with general psychiatric reaction to head injury based on analysis of two hundred cases. *Arch. Neurol. and Psychiat.,* 1945, *53:* 358–364.

KREIG, W. J. S., ARIEFF, A. J., AND WINDLE, W. F. Histological comparison of guinea-pig brains after electric shock and after concussion. *Federation Proc.,* 1945, *4:* 42.

LARGE, A. AND HEINBECKER, P. Nerve degeneration following prolonged cooling of an extremity. *Ann. Surg.,* 1944, *120:* 742–749.

LYNN, J. G., LEVINE, K. N., AND HEWSON, L. R. Psychological tests for the clinical evaluation of late "diffuse organic," "neurotic," and "normal" reactions after closed head injury. *A. Research Nerv. & Ment. Dis., Proc.,* 1945, *24:* 296–378.

MAGOUN, H. W. AND RHINES, R. An inhibitory mechanism in the bulbar reticular formation. *Anat. Rec.,* 1945, *91:* 289.

MEACHAM, W. F., ANGELUCCI, R., BENZ, E., AND PILCHER, C. Chemotherapy of intracranial infections. II. Clinical and pathologic effects of intracranial intro-duction of sulfanilamide, sulfathiazole, and sulfadiazine in normal dogs. *Arch. Neurol. & Psychiat.,* 1943, *50:* 633–651.

MEACHAM, W. F., SMITH, E., AND PILCHER, C. Chemotherapy of intracranial in-fections. V. The treatment of staphylococcic and pneumococcic meningitis with sulfathiazole and sulfadiazine. *War Medicine,* 1944, *6:* 378–381.

MOORE, B. E. AND RUESCH, J. Prolonged disturbances of consciousness following head injury. *New England J. Med.,* 1944, *230:* 445–452.

PILCHER, C. The chemotherapy of intracranial infections. Observations on the use of the sulfonamides and of penicillin under various experimental intracranial conditions. *Ann. Surg.,* 1944, *119:* 509–517.

PILCHER, C., ANGELUCCI, R., AND MEACHAM, W. F. Convulsions produced by the intracranial implantation of sulfathiazole. Preliminary report. *J.A.M.A.,* 1942, *119:* 927.

PILCHER, C. AND MEACHAM, W. F. Absorbable gelatin sponge and thrombin for hemostasis in neurosurgery. Experimental and clinical observations. *Surg., Gynec. & Obst.*, 1945, *81:* 365–369.

PILCHER, C. AND MEACHAM, W. F. The chemotherapy of intracranial infections. III. The treatment of experimental staphylococcic meningitis with intrathecal administration of penicillin. *J.A.M.A.*, 1943, *123:* 330–332.

PILCHER, C. AND MEACHAM, W. F. The chemotherapy of intracranial infections. IV. The treatment of pneumococcal meningitis by intrathecal administration of penicillin. *J. Neurosurg.*, 1944, *1:* 76–81.

POLLOCK, L. J., GOLSETH, J. G., AND ARIEFF, A. J. Changes in chronaxie during degeneration and regeneration of experimentally produced lesions of the sciatic nerve of the cat. *Surg., Gynec. & Obst.*, 1945, *81:* 451–454.

POLLOCK, L. J., GOLSETH, J. G., ARIEFF, A. J., AND MAYFIELD, F. Electrodiagnosis by means of progressive currents of long duration. Studies on peripheral nerve injuries in man. *Surg., Gynec. & Obst.*, 1945, *81:* 192–200.

POLLOCK, L. J., GOLSETH, J. G., AND ARIEFF, A. J. Galvanic tetanus and galvanic tetanus ratio in electrodiagnosis of peripheral nerve lesions. *Surg., Gynec. & Obst.*, 1945, *81:* 660–666.

POLLOCK, L. J., GOLSETH, J. G., AND ARIEFF, A. J. Strength frequency curves in electrodiagnosis of experimentally produced peripheral nerve lesions in the cat. *Surg., Gynec. & Obst.*, 1945, *80:* 235–242.

POLLOCK, L. J., GOLSETH, J. G., AND ARIEFF, A. J. The use of discontinuity of strength duration curves in muscle in diagnosis of peripheral nerve lesions. *Surg., Gynec. & Obst.*, 1944, *79:* 133–141.

POLLOCK, L. J., GOLSETH, J. G., AND ARIEFF, A. J. The use of the height of the whole strength-duration curve of muscle as a diagnostic procedure in experimentally produced lesions of the sciatic nerve in the cat. *Quarterly Bull. Northwestern Univ. Med. School*, 1945, *19:* 169–180.

POLLOCK, L. J., GOLSETH, J. G., ARIEFF, A. J., SHERMAN, I. C., SCHILLER, M. A., AND TIGAY, E. L. Electrodiagnosis by means of progressive currents of long duration. Studies on cats with experimentally produced section of the sciatic nerves. *Arch. Neurol. & Psychiat.*, 1944, *51:* 147–154.

POLLOCK, L. J., GOLSETH, J. G., ARIEFF, A. J., SHERMAN, I. C., SCHILLER, M. A., AND TIGAY, E. L. Reaction of degeneration in electrodiagnosis of experimental peripheral nerve lesions. *War Medicine*, 1945, *7:* 275–283.

RAMBACH, W. A., JR. A study of edema following brain concussion. *Quart. Phi Beta Pi*, 1945, *42:* 119–128.

RAMBACH, W. A., JR., AND RAMIREZ DE ARELLANO, M. ROBERT DE. Alteration in water content of the brain after concussion. *Federation Proc.*, 1945, *4:* 57.

RENAUD, H. Contexts of aggression: Play constructions of head injuries and psychoneurotics. *J. Psychol.*, 1946, *21:* 307–326.

RENAUD, H. Group differences in fantasies: head injuries, psychoneurotics, and brain diseases. *J. Psychol.*, 1946, *21:* 327–346.

RICHTER, C. P. Instructions for using the cutaneous resistance recorder, or "dermometer," on peripheral nerve injuries, sympathectomies, and paravertebral blocks. *J. Neurosurg.*, 1946, *3:* 181–191.

RICHTER, C. P. AND OTENASEK, F. J. Thoracolumbar sympathectomies examined with the electrical skin resistance method. *J. Neurosurg.*, 1946, *3:* 120–134.

RICHTER, C. P. AND WOODRUFF, B. G. Lumbar sympathetic dermatomes in man determined by the electrical skin resistance method. *J. Neurophysiol.*, 1945, *8:* 323–338.

ROBERTS, J. T. Nourishment of cardiac muscles (section of a paper entitled "The role of the small vessels and nerves of the heart in heart failure, coronary artery thrombosis and cardiac pain"). *Med. Ann. Dist. Columbia*, 1945, *24:* 483–495.

RUESCH, J. Dark adaptation, negative after images, tachistoscopic examinations and reaction time in head injuries. *J. Neurosurg.*, 1944, *1:* 243–251.

RUESCH, J. Intellectual impairment in head injuries. *Am. J. Psychiat.*, 1944, *100:* 480–496.

RUESCH, J. Psychophysiological relations in cases with head injuries. *Psychosomatic Medicine*, 1945, *7:* 158–168.

RUESCH, J. AND BOWMAN, K. M. Glucose, insulin, and adrenalin tolerance tests in head injuries. *J. Nerv. & Ment. Dis.*, 1944, *99:* 842–852.

RUESCH, J. AND BOWMAN, K. M. Prolonged post-traumatic syndromes following head injury. *Am. J. Psychiat.*, 1945, *102:* 145–163.

RUESCH, J., HARRIS, R. E., AND BOWMAN, K. M. Pre- and post-traumatic personality in head injuries. *A. Research Nerv. & Ment. Dis., Proc.*, 1945, *24:* 507–539.

RUESCH, J. AND MOORE, B. E. Measurement of intellectual functions in the acute stage of head injury. *Arch. Neurol. & Psychiat.*, 1943, *50:* 165–170.

SCHORR, M. G. AND LAMPORT, H. A portable millivoltmeter. *Yale J. Biol. & Med.*, 1946, *18:* 307–310.

SHAPIRO, A., TARLOV, I. M., OLIVER, R., GOLDFARB, A. I., BOJAR, S., KASLOW, R., AND ROCKENMACHER, M. Plasma clot tensile strength. II. The effect of some physical factors, anticoagulants, and coagulants. *J. Lab. & Clin. Med.*, 1944, *29:* 282–295.

SHEER, C. AND LYNN, J. G. Electronics in the study of head injuries. *Electronics*, 1944, January.

TARLOV, I. M. Autologous plasma clot suture of nerves. Its use in clinical surgery. *J.A.M.A.*, 1944, *126:* 741–748.

TARLOV, I. M. Plasma clot suture of nerves — illustrated technique. *Surgery*, 1944, *15:* 257–269.

TARLOV, I. M. AND BENJAMIN, B. Plasma clot and silk suture of nerves. I. An experimental study of comparative tissue reaction. *Surg., Gynec. & Obst.*, 1943, *76:* 366–374.

TARLOV, I. M., DENSLOW, C., SWARZ, S., AND PINELES, D. Plasma clot suture of nerves: Experimental technic. *Arch. Surg.*, 1943, *47:* 44–58.

TARLOV, I. M. AND EPSTEIN, J. A. Nerve grafts: The importance of an adequate blood supply. *J. Neurosurg.*, 1945, *2:* 49–71.

TARLOV, I. M., GOLDFARB, A. I., AND BENJAMIN, B. A method for measuring the tensile strength and stretch of plasma clots. *J. Lab. & Clin. Med.*, 1942, *27:* 1333–1335.

TAYLOR, A. C. The rates of freezing, drying and dehydration of nerves. *J. Cell. & Comp. Physiol.*, 1945, *25:* 161–173.

ULETT, G. A. AND CLAUSEEN, F. B. A spring-pressure-contact electrode for use in electroencephalographic recording. *Science*, 1944, *99:* 85–86.

WAGREICH, H., SOBEL, A. E., LEVINE, C., AND TARLOV, I. M. Studies on clot strength of dehydrated plasma. *Arch. Biochem.,* 1944, *5:* 233–241.

WAGREICH, H., AND TARLOV, I. M. Studies on the strength of fibrinogen-thrombin clots. *Arch. Biochem.,* 1945, *7:* 345–352.

WALKER, A. E. The syndrome of cerebral concussion. *Clinics,* 1945, *4:* 361–395.

WALKER, A. E., KOLLROS, J. J., AND CASE, T. J. Electroencephalographic alterations following cerebral concussions. *Tr. Am. Neurol. A.,* 1944, *70:* 149–151.

WALKER, A. E., KOLLROS, J. J., AND CASE, T. J. Physiology of concussion. *Arch. Neurol. & Psychiat.,* 1944, *52:* 78–79.

WALKER, A. E., KOLLROS, J. J., AND CASE, T. J. The physiological basis of cerebral concussion. *A. Research Nerv. & Ment. Dis., Proc.,* 1945, *24:* 437–466.

WALKER, A. E., KOLLROS, J. J., AND CASE, T. J. The physiological basis of concussion. *J. Neurosurg.,* 1944, *1:* 103–116.

WEISS, P. Damming of axoplasm in constricted nerve: A sign of perpetual growth in nerve fibers. *Anat. Rec.,* 1944, *88:* 48.

WEISS, P. Endoneurial edema in constricted nerve. *Anat. Rec.,* 1943, *86:* 491–522.

WEISS, P. Evidence of perpetual proximo-distal growth of nerve fibers. *Biol. Bull.,* 1944, *87:* 160.

WEISS, P. Experiments on cell and axon orientation in vitro: The role of colloidal exudates in tissue organization. *J. Exper. Zool.,* 1945, *100:* 353–386.

WEISS, P. Experiments on nerve repair. *Tr. Am. Neurol. A.,* 1943, *69:* 42–45.

WEISS, P. Functional nerve regeneration through frozen-dried nerve grafts in cats and monkeys. *Proc. Soc. Exper. Biol. & Med.,* 1943, *54:* 277–279.

WEISS, P. In vitro transformation of spindle cells of neural origin into macrophages. *Anat. Rec.,* 1944, *88:* 205–221.

WEISS, P. Nerve regeneration in the rat following tubular splicing of severed nerves. *Arch. Surg.,* 1943, *46:* 525–547.

WEISS, P. Nerve reunion with sleeves of frozen-dried artery in rabbits, cats, and monkeys. *Proc. Soc. Exper. Biol. & Med.,* 1943, *54:* 274–277.

WEISS, P. Sutureless reunion of severed nerves with elastic cuffs of tantalum. *J. Neurosurg.,* 1944, *1:* 219–225.

WEISS, P. The technology of nerve regeneration: A review. Sutureless tubulation and related methods of nerve repair. *J. Neurosurg.,* 1944, *1:* 400–450.

WEISS, P. AND BURT, A. S. Effect of nerve compression on Wallerian degeneration in vitro. *Proc. Soc. Exper. Biol. & Med.,* 1944, *55:* 109–112.

WEISS, P. AND CAMPBELL, C. J. Nerve fiber counts and muscle tension after nerve regeneration in the rat. *Am. J. Physiol.,* 1944, *140:* 616–626.

WEISS, P. AND CUMMINGS, J. B. Regeneration of the lateral line nerve of Amblystoma from different nerve fiber sources. *Anat. Rec.,* 1943, *87:* 119–125.

WEISS, P. AND DAVIS, H. Pressure block in nerves provided with arterial sleeves. *J. Neurophysiol.,* 1943, *6:* 269–286.

WEISS, P. AND EDDS, M. V., JR. Sensory-motor nerve crosses in the rat. *J. Neurophysiol.,* 1945, *8:* 173–194.

WEISS, P. AND EDDS, M. V., JR. Spontaneous recovery of muscle following partial denervation. *Am. J. Physiol.,* 1946, *145:* 587–607.

WEISS, P., EDDS, M. V., JR., AND CAVANAUGH, M. The effect of terminal connections on the caliber of nerve fibers. *Anat. Rec.,* 1945, *92:* 215–233.

WEISS, P. AND TAYLOR, A. C. Further experimental evidence against "neurotropism" in nerve regeneration. *J. Exper. Zool.,* 1944, *95:* 233–257.

WEISS, P. AND TAYLOR, A. C. Histomechanical analysis of nerve reunion in the rat after tubular splicing. *Arch. Surg.,* 1943, *47:* 419–447.

WEISS, P. AND TAYLOR, A. C. Impairment of growth and myelinization in regenerating nerve fibers subject to constriction. *Proc. Soc. Exper. Biol. & Med.,* 1944, *55:* 77–80.

WEISS, P. AND TAYLOR, A. C. Repair of peripheral nerves by grafts of frozen-dried nerve. *Proc. Soc. Exper. Biol. & Med.,* 1943, *52:* 326–328.

WEISS, P. AND TAYLOR, A. C. The viability of isolated nerve fragments and its modification by methylene blue. *J. Cell. & Comp. Physiol.,* 1946, *27:* 87–103.

WEISS, P. AND WANG, H. Proximo-distal fluid convection in nerves, demonstrated by color indicators. *Federation Proc.,* 1944, *3:* 51.

WEISS, P. AND WANG, H. Transformation of adult Schwann cells into macrophages. *Proc. Soc. Exper. Biol. & Med.,* 1945, *58:* 273–275.

WEISS, P., WANG, H., TAYLOR, A. C., AND EDDS, M. V., JR. Proximodistal fluid convection in nerves, demonstrated by radioactive tracer substances. *Federation Proc.,* 1944, *3:* 51.

WEISS, P., WANG, H., TAYLOR, A. C., AND EDDS, M. V., JR. Proximodistal fluid convection in the endoneurial spaces of peripheral nerves, demonstrated by colored and radioactive (isotope) tracers. *Am. J. Physiol.,* 1945, *143:* 521–540.

WINDLE, W. F. AND GROAT, R. A. Disappearance of nerve cells after concussion. *Anat. Record,* 1945, *93:* 201–209.

WINDLE, W. F., GROAT, R. A., AND FOX, C. A. Experimental structural alterations in the brain during and after concussion. *Surg., Gynec. & Obst.,* 1944, *79:* 561–572.

WINDLE, W. F., GROAT, R. A., AND FOX, C. A. Structural alterations in the brain in and after experimental concussion. *Federation Proc.,* 1944, *3:* 53.

WINDLE, W. F., GROAT, R. A., AND MAGOUN, H. W. Functional and structural changes in the central nervous system during and after experimental concussion. *Tr. Am. Neurol. A.,* 1944, pp. 117–119.

WINDLE, W. F., KRIEG, W. J. S., AND ARIEFF, A. J. Failure to detect structural changes in the brain after electrical shock. *Quart. Bull. Northwestern Univ. Med. School,* 1945, *19:* 181–188.

WINDLE, W. F., RAMBACH, W. A., JR., RAMIREZ DE ARELLANO, M. I. ROBERT DE, GROAT, R. A., AND BECKER, R. F. Water content of the brain after concussion and its noncontributory relation to the histopathology of concussion. *J. Neurosurg.,* 1946, *3:* 157–164.

WINDLE, W. F., SCHAMP, H. M., AND GROAT, R. A. Destruction and disappearance of brain stem neurons in guinea pigs receiving repeated concussion. *Federation Proc.,* 1945, *4:* 78.

Surgical Specialties

ADAMS, W. E. AND THORNTON, T. F. The use of plasma for filling the pleural space after loss of varying amounts of lung. *Surgery,* 1944, *15:* 521–546.

BERING, E. A., JR. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XX. The development of fibrin foam as a

hemostatic agent and for use in conjunction with human thrombin. *J. Clin. Investigation,* 1944, *23:* 586–590.

BLAKEMORE, A. H. AND LORD, J. W., JR. A nonsuture method of blood vessel anastomosis: Experimental and clinical study. *J.A.M.A.,* 1945, *127:* 685–691 and 748–753.

BLAKEMORE, A. H., LORD, J. W., JR., AND STEFKO, P. L. Restoration of blood flow in damaged arteries: Further studies on a nonsuture method of blood vessel anastomosis. *Ann. Surg.,* 1943, *117:* 481–497.

FRANTZ, V. K. New absorbable hemostatic agents. *Bull. New York Acad. Med.,* 1946, *22:* 102–110.

FRANTZ, V. K. New methods of hemostasis. *Surg. Clin. North America,* 1945, *April:* 338–349.

FRANTZ, V. K., CLARKE, H. T., AND LATTES, R. Hemostasis with absorbable gauze (oxidized cellulose). *Ann. Surg.,* 1944, *120:* 181–198.

FRANTZ, V. K. AND LATTES, R. Oxidized cellulose-absorbable gauze (cellulosic acid). *J.A.M.A.,* 1945, *129:* 798–801.

INGRAHAM, F. D. AND BAILEY, O. T. Clinical use of products of human plasma fractionation. III. The use of products of fibrinogen and thrombin in surgery. *J.A.M.A.,* 1944, *126:* 680–685.

LATTES, R. AND FRANTZ, V. K. Absorbable sponge tests. *Ann. Surg.,* 1945, *121:* 894–896.

LION, K. S. AND SIZER, I. W. Apparatus for measuring rate of enzymatic digestion of absorbable surgical sutures and other protein fibers. *Arch. Surg.,* 1944, *48:* 120–122.

RILEY, R. F., MCCLEARY, B., AND JOHNSON, R. E. Denervation atrophy of bone and muscle. An examination of the effect of choline and some further observations on the metabolism of phosphorylcholine and deposition of P^{32} in bone. *Am. J. Physiol.,* 1945, *143:* 677–686.

SIZER, I. W. The effects of temperature on the digestion of collagen sutures and surgical gut (catgut) by enzymes and by the subcutaneous tissues of the frog. *Ann. Surg.,* 1945, *121:* 231–238.

SMELSER, G. K. AND OZANICS, V. Effect of quick freezing at very low temperatures of donor tissue in corneal transplants. *Proc. Soc. Exper. Biol. & Med.,* 1946, *62:* 274–277.

TAYLOR, A. C. Apparatus for the freezing-drying of tissues for storage. *J. Lab. & Clin. Med.,* 1944, *29:* 657–663.

VOLPITTO, P. P., WOODBURY, R. A., AND ABREU, B. E. Influence of different forms of mechanical artificial respiration on the pulmonary and systemic blood pressure. *J.A.M.A.,* 1944, *126:* 1066–1069.

WEISS, P. The morphogenetic properties of frozen-dried tissues. *Anat. Record,* 1944, *88:* 48.

WEISS, P. AND TAYLOR, A. C. Transplantation of frozen-dried cornea in the rat. *Anat. Record,* 1944, *88:* 49.

WOODBURY, R. A. AND ABREU, B. E. Influence of dying gasps, yawns and sighs on blood pressure and blood flow. *Am. J. Physiol.,* 1944, *142:* 721–726.

Anesthesia

(See PHYSIOLOGY, Shock, for Anesthesia and Shock.)

BEECHER, H. K. An easily transportable apparatus for anesthesia with or without compressed oxygen, especially designed for positive pressure anesthesia in thoracic surgery under military conditions. *War Medicine*, 1942, 2: 602–608.

BEECHER, H. K., BENNETT, H. S., AND BASSETT, D. L. Circulatory effects of increased pressure in the airway. *Anesthesiology*, 1943, 4: 612–618.

BEECHER, H. K. AND MAYER, C. A. Mechanisms of respiratory failure under barbiturate anesthesia. *J. Clin. Investigation*, 1941, 20: 549–566.

CORCORAN, A. C. AND PAGE, I. H. Effects of anesthetic dosage of pentobarbital sodium on renal function and blood pressure in dogs. *Am. J. Physiol.*, 1943, 140: 234–239.

FORBES, J. C. AND EVANS, E. I. Protective action of sulfanilamide against hepatic damage from chloroform inhalation. *War Medicine*, 1943, 4: 418–421.

WHITE, J. C., VERLOT, M., SELVERSTONE, B., AND BEECHER, H. K. Changes in brain volume during anesthesia: The effects of anoxia and hypercapnia. *Arch. Surg.*, 1942, 44: 1–21.

X-ray Procedures

HODGES, P. C. AND MORGAN, R. H. Photoelectric timing in general roentgenography. *Am. J. Roentgenol.*, 1945, 53: 474–482.

MORGAN, R. H. A quantitative study of the effect of temperature on sensitivities of x-ray screens and films. *Radiology*, 1944, 43: 256–266.

MORGAN, R. H. A simple method for measuring peak voltage in diagnostic roentgen equipment. *Am. J. Roentgenol.*, 1944, 52: 208–211.

MORGAN, R. H. Reciprocity law failure in x-ray films. *Radiology*, 1944, 42: 471–479.

MORGAN, R. H. The automatic control of exposure in photofluorography. *Pub. Health Rep.*, 1943, 58: 1533–1541.

AVIATION MEDICINE

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness: I. The effects of variation of time intervals between accelerations upon sickness rates. *J. Psychol.*, 1945, 19: 49–62.

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness. II. A second approach to the problem of the effects of variation of time intervals between accelerations upon sickness rates. *J. Psychol.*, 1945, 19: 63–68.

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness. III. The effects of various accelerations upon sickness rates. *J. Psychol.*, 1945, 20: 3–8.

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness. IV. The effects of waves containing two acceleration levels upon sickness. *J. Psychol.*, 1945, 20: 9–18.

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness. V. Incidence of sickness at various hours of the day. *J. Psychol.*, 1945, *20:* 19–24.

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness. VI. Prediction of sickness on a vertical accelerator by means of a motion sickness history questionnaire. *J. Psychol.*, 1945, *20:* 25–30.

ALEXANDER, S. J., COTZIN, M., HILL, C. J., JR., RICCIUTI, E. A., AND WENDT, G. R. Wesleyan University studies of motion sickness. VII. The effects of sickness upon performance. *J. Psychol.*, 1945, *20:* 31–39.

ALLEN, S. C., TAYLOR, C. L., AND HALL, V. E. A study of orthostatic insufficiency by the tiltboard method. *Am. J. Physiol.*, 1945, *143:* 11–20.

BALDES, E. J. AND PORTER, A. N. Human centrifuge for use in studies of man's reaction to acceleration. *Federation Proc.*, 1945, *4:* 4.

BALDES, E. J. AND PORTER, A. N. Human centrifuge operation (motion picture). *Federation Proc.*, 1945, *4:* 3–4.

BARACH, A. L. Aviation Medicine. Russell L. Cecil's *Textbook of Medicine,* Philadelphia, W. B. Saunders Co., *6th Edition,* 1943, pp. 495–503.

BARACH, A. L. Aviation Medicine. Nelson's Loose-leaf *Living Med.,* Thomas Nelson & Sons, New York, 1944, *2:* 673–676A.

BARACH, A. L. The treatment of pulmonary edema due to gas poisoning in war and in civilian life with special reference to use of positive-pressure respiration. *New England J. Med.*, 1944, *230:* 216–223.

BARACH, A. L., BROOKES, R., ECKMAN, M., GINSBURG, E., AND JOHNSON, A. E. Appraisal of tests of altitude tolerance. *J. Aviation Med.*, 1943, *14:* 55–62.

BARACH, A. L., BROOKES, R., ECKMAN, M., GINSBURG, E., AND JOHNSON, A. E. Impairment of emotional control as a test of altitude anoxia. *J. Aviation Med.*, 1943, *14:* 63–70.

BARACH, A. L., ECKMAN, M., OPPENHEIMER, E. T., RUMSEY, C., JR., AND SOROKA, M. Observations on methods of increasing resistance to oxygen poisoning and studies of accompanying physiological effects. *Am. J. Physiol.*, 1944, *142:* 462–475.

BARACH, A. L., JOHNSON, A. E., AND RULE, C. Development of active pulmonary tuberculosis as the probable result of decompression sickness at simulated altitudes above 40,000 feet. *J. Aviation Med.*, 1945, *16:* 152–155.

BARACH, A. L., MOLOMUT, N., AND LANDY, S. The effect of preoxygenation on newborn rats exposed to a simulated altitude of 55,000 feet (barometric pressure of 67.8 mm. Hg); preliminary report. *J. Aviation Med.*, 1942, *13:* 190–192.

BATEMAN, J. B. Factors influencing the composition of alveolar air in normal persons. *Proc. Staff Meet. Mayo Clin.*, 1945, *20:* 213–223.

BATEMAN, J. B. Notes on a modified open-circuit method for the measurement of residual air. *Proc. Staff Meet. Mayo Clin.*, 1945, *20:* 482–485.

BERG, W. E., HARRIS, M., WHITAKER, D. M., AND TWITTY, V. C. Additional mechanisms for the origin of bubbles in animals decompressed to simulated altitudes. *J. Gen. Physiol.*, 1945, *28:* 253–258.

BLANKENHORN, M. A. AND FERRIS, E. B., JR. On the nature of aviators' bends. *Tr. A. Am. Physicians*, 1944, *58:* 86–91.

BOOTHBY, W. M. Effects of high altitudes on the composition of alveolar air. Introductory remarks. *Proc. Staff Meet. Mayo Clin.,* 1945, *20:* 209–213.

BRIDGE, E. V., HENRY, F. M., COOK, S. F., WILLIAMS, O. L., LYONS, W. R., AND LAWRENCE, J. H. Decompression sickness: Nature and incidence of symptoms during and after artificial decompression to 38,000 feet for ninety minutes with exercise during exposure. *J. Aviation Med.,* 1944, *15:* 316–327.

BRIDGE, E. V., HENRY, F. M., AND LAWRENCE, J. H. Observations of multiple joint pains in the extremities at 38,000 feet. *J. Nerv. & Ment. Dis.,* 1945, *101:* 583–588.

BRIDGE, E. V., HENRY, F. M., WILLIAMS, O. L., AND LAWRENCE, J. H. "Chokes": A respiratory manifestation of aeroembolism in high altitude flying. *Ann. Int. Med.,* 1945, *22:* 398–407.

BRIEGER, H. Carbon monoxide polycythemia. *J. Indust. Hyg. & Toxicol.,* 1944, *26:* 321–327.

BRITTON, S. W., COREY, E. L., AND STEWART, G. A. Effects of high acceleratory forces and their alleviation. *Am. J. Physiol.,* 1946, *146:* 33–51.

BROWN, S. C., GOOD, W. M., AND EVANS, R. D. An atmospheric pressure Geiger-Müller counter system for the study of respiratory problems. *Rev. Scient. Instruments,* 1945, *16:* 125–129.

BRYAN, A. H. AND RICKETTS, H. T. The effect in man of chronic intermittent anoxia on glucose tolerance and the urinary excretion of sodium, potassium, chloride, phosphorus, nitrogen, and 17-ketosteroids. *J. Clin. Endocrinology,* 1944, *4:* 450–464.

BURRILL, M. W., FREEMAN, S., AND IVY, A. C. Sodium, potassium, and chloride excretion of human subjects exposed to a simulated altitude of eighteen thousand feet. *J. Biol. Chem.,* 1945, *157:* 297–302.

CLARKE, R. W., MARSHALL, C., AND NIMS, L. F. Blood pH during decompression. *Am. J. Physiol.,* 1944, *142:* 483–486.

CLINTON, M., JR. AND THORN, G. W. Studies on commercial air line pilots. *War Medicine,* 1943, *4:* 363–373.

CODE, C. F., WOOD, E. H., AND BALDES, E. J. Hydrostatic anti-blackout protection; the protection afforded man against the effects of positive acceleration by immersion in water (motion picture). *Federation Proc.,* 1945, *4:* 15.

CODE, C. F., WOOD, E. H., STURM, R. E., LAMBERT, E. H., AND BALDES, E. J. The sequence of physiologic events in man during exposure to positive acceleration. *Federation Proc.,* 1945, *4:* 14–15.

COMROE, J. H., JR. AND DRIPPS, R. D., JR. The oxygen tension of arterial blood and alveolar air in normal human subjects. *Am. J. Physiol.,* 1944, *142:* 700–707.

COMROE, J. H., JR., DRIPPS, R. D., DUMKE, P. R., AND DEMING, M. Oxygen toxicity. The effect of inhalation of high concentrations of oxygen for twenty-four hours on normal men at sea level and at a simulated altitude of 18,000 feet. *J.A.M.A.,* 1945, *128:* 710–717.

COOK, S. F. The inhibition of animal metabolism under decompression. *J. Aviation Med.,* 1945, *16:* 268–271.

COOK, S. F. AND SEARS, W. N. Studies on the cardiovascular system of dogs with radioactive inert gases. *Am. J. Physiol.,* 1945, *144:* 164–167.

COOK, S. F. AND STRAJMAN, E. The effect of decompression on human metabolism during and after exercise. *Am. J. Physiol.,* 1945, *144:* 637–642.

Cook, S. F., Williams, O. L., Lyons, W. R., and Lawrence, J. H. A comparison of altitude and exercise with respect to decompression sickness. *War Medicine,* 1944, *6:* 182–187.

Dean, R. B. The formation of bubbles. *J. Applied Physics,* 1944, *15:* 446–451.

DeHaven, H. Causes of injury in light plane accidents. *Aero Digest,* 1944, *Mar. 1:* 51–56.

DeHaven, H. Mechanics of injury under force conditions. *Mechanical Engineering,* 1944, *April:* 264–268.

Dorrance, S. S., Thorn, G. W., Clinton, M., Jr., Edmonds, H. W., and Farber, S. Effect of cobalt on work performance under conditions of anoxia. *Am. J. Physiol.,* 1943, *139:* 399–405.

Dorrance, S. S., Thorn, G. W., Tyler, F. H., and Katzin, B. Work performance of normal rats under conditions of anoxia. *Endocrinology,* 1942, *31:* 209–216.

Drabkin, D. L. and Schmidt, C. F. Spectrophotometric studies. XII. Observations of circulating blood in vivo, and the direct determination of the saturation of hemoglobin in arterial blood. *J. Biol. Chem.,* 1945, *157:* 69–83.

Du Bois, E. F. The anatomy and physiology of the airplane cockpit. *Aeronaut. Engineering Rev.,* 1945, *4:* 1.

Eckman, M. and Barach, A. L. A combined mixing and flow regulator. *J. Aviation Med.,* 1945, *16:* 265–267.

Eckman, M. and Barach, A. L. The effect on the vital capacity of a swift ascent to a simulated altitude of 35,000 feet. *J. Aviation Med.,* 1942, *13:* 36–42.

Edelmann, A., Whitehorn, W. V., and Hitchcock, F. A. The effect of breathing 100% oxygen on ventilation volume and alveolar CO_2 tension. *Federation Proc.,* 1945, *4:* 18.

Ehrich, W. E., Bellet, S., and Lewey, F. H. Cardiac changes from CO poisoning. *Am. J. M. Sc.,* 1944, *208:* 511–523.

Engel, G. L., Ferris, E. B., Webb, J. P., and Stevens, C. D. Studies on voluntary breath-holding. *Proc. Central Soc. Clin. Res.,* Chicago, 1944, *17:* 61.

Engel, G. L., Webb, J. P., and Ferris, E. B. Quantitative electroencephalographic studies of anoxia in humans; comparison with acute alcoholic intoxication and hypoglycemia. *J. Clin. Investigation,* 1945, *24:* 691–697.

Engel, G. L., Webb, J. P., Ferris, E. B., Jr., Romano, J., Ryder, H., and Blankenhorn, M. A. A migraine-like syndrome complicating decompression sickness: Scintillating scotomas, focal neurologic signs and headache; clinical and electroencephalographic observations. *War Medicine,* 1944, *5:* 304–314.

Fulton, J. F. and Hoff, E. C. A five-foot shelf on aviation medicine. *Bull. M. Library A.,* 1942, *30:* 368–371.

Fulton, J. F., Hoff, P. M., and Perkins, H. T. A bibliography of visual literature 1939–1944. Menasha, Wis., George Banta Publishing Co., 1945, 201 pages.

Halstead, W. C. Chronic intermittent anoxia and the dynamic visual field. *J. Psychol.,* 1945, *20:* 49–56.

Harris, M., Berg, W. E., Whitaker, D. M., and Twitty, V. C. The relation of exercise to bubble formation in animals decompressed to sea level from high barometric pressures. *J. Gen. Physiol.,* 1945, *28:* 241–251.

Harris, M., Berg, W. E., Whitaker, D. M., Twitty, V. C., and Blinks, L. R. Carbon dioxide as a facilitating agent in the initiation and growth of bubbles

in animals decompressed to simulated altitudes. *J. Gen. Physiol.,* 1945, *28:* 225–240.

HARVEY, E. N. Decompression sickness and bubble formation in blood and tissues. *Bull. New York Acad. Med.,* 1945, *21:* 505–536.

HARVEY, E. N., BARNES, D. K., McELROY, W. D., WHITELEY, A. H., AND PEASE, D. C. Removal of gas nuclei from liquids and surfaces. *J. Am. Chem. Soc.,* 1945, *67:* 156.

HARVEY, E. N., BARNES, D. K., McELROY, W. D., WHITELEY, A. H., PEASE, D. C., AND COOPER, K. W. Bubble formation in animals. I. Physical factors. *J. Cell & Comp. Physiol.,* 1944, *24:* 1–22.

HARVEY, E. N., McELROY, W. D., WHITELEY, A. H., WARREN, G. H., AND PEASE, D. C. Bubble formation in animals. III. An analysis of gas tension and hydrostatic pressure in cats. *J. Cell & Comp. Physiol.,* 1944, *24:* 117–132.

HARVEY, E. N., WHITELY, A. H., McELROY, W. D., PEASE, D. C., AND BARNES, D. K. Bubble formation in animals. II. Gas nuclei and their distribution in blood and tissues. *J. Cell. & Comp. Physiol.,* 1944, *24:* 23–34.

HELMHOLZ, H. F., JR. Effect of altitude, added oxygen and pressure breathing on transportation of oxygen by the blood. *Proc. Staff Meet. Mayo Clin.,* 1945, *20:* 223–229.

HELMHOLZ, H. F., JR., BATEMAN, J. B., AND BOOTHBY, W. M. The effects of altitude anoxia on the respiratory processes. *J. Aviation Med.,* 1944, *15:* 366–380.

HENRY, F. M. Altitude pain: A study of individual differences in susceptibility to bends, chokes, and related symptoms. *J. Aviation Med.,* 1946, *17:* 28–55.

HENRY, F. M. The role of exercise in altitude pain. *Am. J. Physiol.,* 1946, *145:* 279–284.

HENRY, F. M. AND COOK, S. F. Effectiveness of pre-flight oxygen breathing in preventing decompression sickness. *J. Aviation Med.,* 1945, *16:* 350–355.

HIMWICH, H. E., FAZEKAS, J. F., HERRLICH, H. C., JOHNSON, A. E., AND BARACH, A. L. Studies on effects of adding carbon dioxide to oxygen-enriched atmospheres in low pressure chambers, oxygen and carbon dioxide tensions of cerebral blood. *J. Aviation Med.,* 1942, *13:* 177–181.

HOFF, E. C. AND FULTON, J. F. A bibliography of aviation medicine. Springfield, Ill., and Baltimore, Md., Charles C. Thomas, 1942, 240 pages (5745 entries).

HOFF, E. C. AND YAHN, C. Effect of sodium 5,5-diphenyl hydantoinate (dilantin sodium) upon the tolerance of rats and mice to decompression. *Am. J. Physiol.,* 1944, *141:* 7–16.

HOFF, P. M., HOFF, E. C., AND FULTON, J. F. A bibliography of aviation medicine: supplement. Menasha, Wis., George Banta Publishing Co., 109 pages.

KETY, S. S. AND SCHMIDT, C. F. The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. *Am. J. Physiol.,* 1945, *143:* 53–66.

KING, C. G., BICKERMAN, H. A., BOUVET, W., HARRER, C. J., OYLER, J. R., AND SEITZ, C. P. Aviation nutrition studies. I. Effects of pre-flight and in-flight meals of varying composition with respect to carbohydrate, protein, and fat. *J. Aviation Med.,* 1945, *16:* 69–84.

KING, C. G., BOUVET, W., COOK, M. N., HARRER, C. J., OYLER, J. R., AND SCHWIMMER, D. Observations on the effect of pre-flight meals upon altitude tolerance. *Science,* 1945, *102:* 36–38.

LAMBERT, E. H. The physiologic basis of "blackout" as it occurs in aviators. *Federation Proc.*, 1945, *4*: 43.

LAMBERT, E. H., HALLENBECK, G. A., BALDES, E. J., WOOD, E. H., AND CODE, C. F. The symptoms which occur in man during exposure to positive acceleration (motion picture). *Federation Proc.*, 1945, *4*: 43.

LAMPORT, H., HOFF, E. C., AND HERRINGTON, L. P. Statistically valid tests of drugs and other factors affecting the resistance of mice to acceleration. *Am. J. Physiol.*, 1945, *143*: 262–271.

LAWRENCE, J. H. Aviation medical problems with special reference to altitude pain. *J. Nerv. & Ment. Dis.*, 1944, *99*: 703–711.

LAWRENCE, J. H., AND LUND, D. W. Two severe altitude flight reactions. *Navy Department's BuMed News Letter, Aviation Supplement*, 1945, *4*: 3–5.

LAWRENCE, J. H., TOBIAS, C. R., LYONS, W. R., HELMHOLZ, H. F., JR., SWEENEY, A. R., JR., BRIDGE, E. V., AND WILLIAMS, O. L. A study of aeromedical problems in a Liberator bomber at high altitude. *J. Aviation Med.*, 1945, *16*: 286–310.

LEWEY, F. H., AND DRABKIN, D. L. Experimental chronic carbon monoxide poisoning of dogs. *Am. J. M. Sc.*, 1944, *208*: 502–511.

LINDSAY, J. R., OPPENHEIMER, M. J., WYCIS, H. T., AND SPIEGEL, E. A. Receptor apparatus of the vestibulovasomotor reaction. *Arch. Otolaryng.*, 1945, *42*: 257–266.

LOW, F. N. The peripheral visual acuity of 100 subjects under scotopic conditions. *Am. J. Physiol.*, 1946, *146*: 21–25.

LUND, D. W., LAWRENCE, J. H., AND LAWRENCE, L. B. Latent neurologic manifestations following decompression: Report of a case of severe reaction following ascent to 38,000 feet. *Occup. Med.*, 1946, *1*: 75–80.

LURIE, M. H., DAVIS, H., AND HAWKINS, J. E. Acoustic trauma of the organ of Corti in the guinea pig. *Laryngoscope*, 1944, *54*: 375–386.

McELROY, W. D., WHITELEY, A. H., COOPER, K. W., PEASE, D. C., WARREN, G. H., AND HARVEY, E. N. Bubble formation in animals. VI. Physiological factors: The role of circulation and respiration. *J. Cell. & Comp. Physiol.*, 1944, *24*: 273–290.

McELROY, W. D., WHITELEY, A. H., WARREN, G. H., AND HARVEY, E. N. Bubble formation in animals. IV. The relative importance of carbon dioxide concentration and mechanical tension during muscle contraction. *J. Cell. & Comp. Physiol.*, 1944, *24*: 133–146.

MILLIKAN, G. A. The oximeter, an instrument for measuring continuously the oxygen saturation of arterial blood in man. *Rev. Scient. Instruments*, 1942, *13*: 434–444.

MORITZ, A. R., AND WEISIGER, J. R. Effects of cold air on the air passages and lungs: An experimental investigation. *Arch. Int. Med.*, 1945, *75*: 233–240.

MORRISON, L. R. Histopathological effect of anoxia on the central nervous system. *Arch. Neurol. & Psychiat.*, 1946, *55*: 1–34.

OTIS, A. B., RAHN, H., EPSTEIN, M. A., AND FENN, W. O. Performance as related to composition of alveolar air. *Am. J. Physiol.*, 1946, *146*: 207–221.

RAHN, H., OTIS, A. B., CHADWICK, L. E., AND FENN, W. O. The pressure-volume diagram of the thorax and lung. *Am. J. Physiol.*, 1946, *146*: 161–178.

RASBERRY, E. A. JR. The effect of succinylsulfathiazole on the volume of intestinal gas in normal young men. *Gastroenterol.*, 1945, *5:* 175–180.

RICKETTS, H. T., ADAMS, W. R., ALVING, A. S., BAY, E. B., BRYAN, A. H., CARMICHAEL, H. T., CASE, T. J., HALSTEAD, W. C., AND LANDOWNE, M. The effect of mild anoxia on neuromuscular co-ordination in skilled investigators. *J. Aviation Med.*, 1945, *16:* 429–431.

RIGGS, B. C. Cyanide loss from media in studies of tissue metabolism in vitro. *J. Biol. Chem.*, 1945, *161:* 381–387.

RIGGS, B. C. The effect of exposure to oxygen at high pressure upon the tonus and respiration of pyloric muscle from the rabbit. *Am. J. Physiol.*, 1945, *145:* 211–217.

ROMANO, J., ENGEL, G. L., FERRIS, E. B. JR., RYDER, H. W., WEBB, J. P., AND BLANKENHORN, M. A. Problems of fatigue as illustrated by experiences in the decompression chamber. *War Medicine*, 1944, *6:* 102–105.

ROMANO, J., ENGEL, G. L., WEBB, J. P., FERRIS, E. B., RYDER, H. W., AND BLANKENHORN, M. A. Syncopal reactions during simulated exposure to high altitude in decompression chamber. *War Medicine*, 1943, *4:* 475–489.

ROUGHTON, F. J. W., AND ROOT, W. S. The estimation of small amounts of carbon monoxide in blood. *J. Biol. Chem.*, 1945, *160:* 123–133.

ROUGHTON, F. J. W., AND ROOT, W. S. The fate of carbon monoxide in the body. *Federation Proc.*, 1945, *4:* 61–62.

ROUGHTON, F. J. W., AND ROOT, W. S. The fate of CO in the body during recovery from mild carbon monoxide poisoning in man. *Am. J. Physiol.*, 1945, *145:* 239–252.

RUSSELL, W. E., ERWIN, J. R., AND DEHAVEN, H. Medical research in some aspects of aircraft design. *J. Aeronautical Sc.*, 1943, *10:* 227–231.

SCHMIDT, C. F., KETY, S. S., AND PENNES, H. H. The gaseous metabolism of the brain of the monkey. *Am. J. Physiol.*, 1945, *143:* 33–52.

SHEARD, C. Effects of anoxia, oxygen, and increased intrapulmonary pressure on dark adaptation. *Proc. Staff Meet. Mayo Clin.*, 1945, *20:* 229–236.

SPIEGEL, E. A. Depressor effect of cold upon the static receptors of the labyrinth. *Federation Proc.*, 1944, *3:* 44.

SPIEGEL, E. A. Depressor effects of cold upon static receptors of the labyrinth. *Am. J. Physiol.*, 1944, *141:* 404–409.

SPIEGEL, E. A. Production of labyrinthine paralysis by application of local anesthetics to external and middle ear. *Proc. Soc. Exper. Biol. & Med.*, 1944, *56:* 137–139.

SPIEGEL, E. A., OPPENHEIMER, M. J., HENNY, G. C., AND WYCIS, H. T. Experimental production of motion sickness. *War Medicine*, 1944, *6:* 283–290.

SPIEGEL, E. A., OPPENHEIMER, M. J., AND WYCIS, H. T. The receptor mechanism of vestibulo-vasomotor reactions induced by rotation. *Federation Proc.*, 1944, *3:* 44.

STADIE, W. C., AND HAUGAARD, N. Oxygen poisoning. V. The effect of high oxygen pressures upon enzymes: Succinic dehydrogenase and cytochrome oxidase. *J. Biol. Chem.*, 1945, *161:* 153–174.

STADIE, W. C., AND HAUGAARD, N. Oxygen poisoning. VII. The effect of high oxygen pressure upon enzymes: Uricase, xanthine oxidase, and d-amino acid oxidase. *J. Biol. Chem.*, 1945, *161:* 181–188.

STADIE, W. C., AND RIGGS, B. C. An apparatus for the determination of the gaseous metabolism of surviving tissues in vitro at high pressures of oxygen. *J. Biol. Chem.*, 1944, *154:* 669–686.

STADIE, W. C., AND RIGGS, B. C. Microtome for the preparation of tissue slices for metabolic studies of surviving tissues in vitro. *J. Biol. Chem.*, 1944, *154:* 687–690.

STADIE, W. C., RIGGS, B. C., AND HAUGAARD, N. Oxygen poisoning. *Am. J. M. Sc.*, 1944, *207:* 84–114.

STADIE, W. C., RIGGS, B. C., AND HAUGAARD, N. Oxygen poisoning. III. The effect of high oxygen pressures upon the metabolism of brain. *J. Biol. Chem.*, 1945, *160:* 191–208.

STADIE, W. C., RIGGS, B. C., AND HAUGAARD, N. Oxygen poisoning. IV. The effect of high oxygen pressures upon the metabolism of liver, kidney, lung, and muscle tissue. *J. Biol. Chem.*, 1945, *160:* 209–216.

STADIE, W. C., RIGGS, B. C., AND HAUGAARD, N. Oxygen poisoning. VI. The effect of high oxygen pressure upon enzymes: Pepsin, catalase, cholinesterase, and carbonic anhydrase. *J. Biol. Chem.*, 1945, *161:* 175–180.

STADIE, W. C., RIGGS, B. C., AND HAUGAARD, N. Oxygen poisoning. VIII. The effect of high oxygen pressure on enzymes: The system synthesizing acetylcholine. *J. Biol. Chem.*, 1945, *161:* 189–196.

STEVENS, C. D., AND SMITH, C. C. Simple apparatus for the study of blood-gas equilibria. *Arch. Biochem.*, 1944, *5:* 357–363.

STEVENS, C. D., VAN FOSSEN, P., FRIEDLANDER, J. K., RATTERMANN, B. J., AND INATOME, M. Oxygen-absorbing solution for the Scholander quick gas analyzer. *Indust. & Engin. Chem.*, 1945, *17:* 598.

STURM, R. E., WOOD, E. H., AND LAMBERT, E. H. Determination of man's blood pressure on the human centrifuge during positive acceleration. *Federation Proc.*, 1945, *4:* 69.

TAYLOR, C., AND BROWN, G. E. JR. — see PHYSIOLOGY, Miscellaneous Physiological Studies.

THOMAS, S. F., AND WILLIAMS, O. L. High-altitude joint pains (bends): Their roentgenographic aspects. *Radiology*, 1945, *44:* 259–261.

THORN, G. W., CLINTON, M. JR., DAVIS, B. M., AND LEWIS, R. A. Effect of adrenal cortical hormone therapy on altitude tolerance. *Endocrinology*, 1945, *36:* 381–390.

THORN, G. W., JONES, B. F., LEWIS, R. A., MITCHELL, E. R., AND KOEPF, G. F. The role of the adrenal cortex in anoxia: The effect of repeated daily exposures to reduced oxygen pressure. *Am. J. Physiol.*, 1942, *137:* 606–619.

TOBIAS, C. A., LAWRENCE, J. H., ROUGHTON, F. J. W., ROOT, W. S., AND GREGERSEN, M. K. The elimination of carbon monoxide from the human body with reference to the possible conversion of CO to CO_2. *Am. J. Physiol.*, 1945, *145:* 253–263.

TOBIAS, C. A., LYONS, W. R., WILLIAMS, O. L., BRIDGE, E. V., LAWRENCE, J. H., HELMHOLZ, H. F. JR., AND SWEENEY, A. R. JR. Study of bends: A comparison in low pressure chamber and actual flight. *Air Surg. Bull.* 1944, *1:* 10.

TYLER, D. B. The influence of a placebo, body position and medication on motion sickness. *Am. J. Physiol.*, 1946, *146:* 458–466.

WARDEN, C. J., AND BROWN, H. C. A preliminary investigation of form and motion acuity at low levels of illumination. *J. Exper. Psychol.,* 1944, *34:* 437–449.

WARDEN, C. J., BROWN, H. C., AND ROSS, S. A study of individual differences in motion acuity at scotopic levels of illumination. *J. Exper. Psychol.,* 1945, *35:* 57–70.

WARDEN, C. J., AND ROSS, S. A comparison of the Navy Adaptometer Test and the Columbia Motion Acuity Test. *J. Exper. Psychol.,* 1945, *35:* 147–152.

WEYMOUTH, F. W., AND HIRSCH, M. J. The reliability of certain tests for determining distance discrimination. *Am. J. Psychol.,* 1945, *58:* 379–390.

WHITAKER, D. M., BLINKS, L. R., BERG, W. E., TWITTY, V. C., AND HARRIS, M. Muscular activity and bubble formation in animals decompressed to simulated altitudes. *J. Gen. Physiol.,* 1945, *28:* 213–223.

WHITEHORN, W. V., EDELMANN, A., AND HITCHCOCK, F. A. The cardiovascular responses to the breathing of 100 per cent oxygen at normal barometric pressure. *Am. J. Physiol.,* 1946, *146:* 61–65.

WHITEHORN, W. V., EDELMANN, A., AND HITCHCOCK, F. A. The effect of inhalation of 100% oxygen on cardiac output in man. *Federation Proc.,* 1945, *4:* 76.

WHITELY, A. H., McELROY, W. D., WARREN, G. H., AND HARVEY, E. N. Bubble formation in animals. V. Denitrogenation. *J. Cell. & Comp. Physiol.,* 1944, *24:* 257–271.

WOOD, E. H., BALDES, E. J., AND CODE, C. F. The external appearance of the human being during positive acceleration. *Air Surgeon's Bull.,* 1945, *2:* 117.

WOOD, E. H., CLARK, D. M., AND LAMBERT, E. H. An analysis of factors involved in the protection afforded man by pneumatic anti-blackout suits. *Federation Proc.,* 1945, *4:* 79.

WOOD, E. H., AND HALLENBECK, G. A. Voluntary (self-protective) maneuvers which can be used to increase man's tolerance to positive acceleration (motion picture). *Federation Proc.,* 1945, *4:* 78–79.

PHYSIOLOGY

Shock

ALEXANDER, B., LANDWEHR, G., AND SELIGMAN, A. M. A specific micromethod for the colorimetric determination of glycine in blood and urine. *J. Biol. Chem.,* 1945, *160:* 51–59.

ALEXANDER, B., AND SELIGMAN, A. M. A colorimetric method for the microdetermination of alpha-alanine in blood. *J. Biol. Chem.,* 1945, *159:* 9–19.

ALLISON, J. B., COLE, W. H., WALCOTT, W. W., GALFAN, S., ROOT, W. S., AND GREGERSEN, M. I. The objective evaluation of therapy in hemorrhagic shock. *Federation Proc.,* 1945, *4:* 2–3.

AUB, J. C. A toxic factor in experimental traumatic shock. *New England J. Med.,* 1944, *231:* 71–75.

AUB, J. C., BRUES, A. M., DUBOS, R., KETY, S. S., NATHANSON, I. T., POPE, A., AND ZAMECNIK, P. C. Bacteria and the "toxic factor" in shock. *War Medicine,* 1944, *5:* 71–73.

Aub, J. C., Brues, A. M., Kety, S. S., Nathanson, I. T., Nutt, A. L., Pope, A., and Zamecnik, P. C. The toxic factors in experimental traumatic shock. IV. The effects of the intravenous injection of the effusion from ischemic muscle. *J. Clin. Investigation*, 1945, *24:* 845–849.

Beatty, C. H. The ability of the liver to change blood glucose and lactate concentrations following severe hemorrhage. *Am. J. Physiol.,* 1945, *144:* 233–238.

Beatty, C. H. The effect of hemorrhage on the lactate/pyruvate ratio and arterial-venous differences in glucose and lactate. *Am. J. Physiol.,* 1945, *143:* 579–588.

Beecher, H. K. The choice of anesthesia for seriously wounded patients. *J.A.M.A.,* 1943, *121:* 899–903.

Beecher, H. K., and Craig, F. N. Tissue metabolism following shock induced by hemorrhage. *J. Biol. Chem.,* 1943, *148:* 383–392.

Beecher, H. K., and McCarrell, J. D. Reduction of fluid loss from damaged (burned) tissues by a barbiturate. *J. Pharmacol. & Exper. Therap.,* 1943, *78:* 39–48.

Beecher, H. K., McCarrell, J. D., and Evans, E. I. A study of the "shock-delaying" action of the barbiturates, with a consideration of the failure of oxygen-rich atmospheres to delay the onset of experimental shock during anesthesia. *Ann. Surg.,* 1942, *116:* 658–667.

Bennett, H. S., Bassett, D. L., and Beecher, H. K. Influence of anesthesia (ether, cyclopropane, sodium evipal) on the circulation under normal and shock conditions. *J. Clin. Investigation,* 1944, *23:* 181–208.

Blalock, A. Effects of continuous and of intermittent application of a tourniquet to a traumatized extremity. *Arch. Surg.,* 1944, *48:* 489–490.

Blalock, A. Effects of lowering temperature of an injured extremity to which a tourniquet has been applied. *Arch. Surg.,* 1943, *46:* 167–170.

Blalock, A. Effects of morphine in experimental shock due to hemorrhage. *Arch. Surg.,* 1943, *47:* 326–328.

Blalock, A. A study of thoracic duct lymph in experimental crush injury and injury produced by gross trauma. *Bull. Johns Hopkins Hosp.,* 1943, *72:* 54–61.

Blalock, A. The utilization of oxygen by the brain in traumatic shock. *Arch. Surg.,* 1944, *49:* 167–169.

Blalock, A., and Duncan, G. W. Traumatic shock — a consideration of several types of injuries. *Surg., Gynec. & Obst.,* 1942, *75:* 401–409.

Blalock, A., and Price, P. B. Panel discussions; traumatic shock — early signs, prevention, and treatment. *Bull. Am. Coll. Surgeons,* 1942, *27:* 102–105.

Brannon, E. S., Stead, E. A., Jr., Warren, J. V., and Merrill, A. J. Hemodynamics of acute hemorrhage in man. *Am. Heart J.,* 1946, *31:* 407–412.

Brues, A. M., Cohn, W. E., Kety, S. S., Nathanson, I. T., Nutt, A. L., Tibbetts, D. M., Zamecnik, P. C., and Aub, J. C. The toxic factors in experimental traumatic shock. II. Studies on electrolyte and water balance in shock. *J. Clin. Investigation,* 1945, *24:* 835–838.

Burdette, W. J., and Wilhelmi, A. E. Respiration of heart muscle slices from rats in the terminal stage of hemorrhagic shock. *Proc. Soc. Exper. Biol. & Med.,* 1946, *61:* 411–413.

Cashman, C. W., Jr. An operative technic for the ligation of the blood supply to the brain in dogs. *Yale J. Biol. & Med.,* 1945, *17:* 461–463.

CHAMBERS, R., AND ZWEIFACH, B. W. Topography and function of the mesenteric capillary circulation. *Am. J. Anat.*, 1944, *75:* 173–205.

CHAMBERS, R., ZWEIFACH, B. W., AND LOWENSTEIN, B. E. Circulatory reactions of rats traumatized in the Noble-Collip drum. *Am. J. Physiol.*, 1943, *139:* 123–128.

CHAMBERS, R., ZWEIFACH, B. W., AND LOWENSTEIN, B. E. The peripheral circulation during the tourniquet shock syndrome in the rat. *Ann. Surg.*, 1944, *120:* 791–802.

CHAMBERS, R., ZWEIFACH, B. W., LOWENSTEIN, B. E., AND LEE, R. E. Vaso-excitor and -depressor substances as "toxic" factors in experimentally induced shock. *Proc. Soc. Exper. Biol. & Med.*, 1944, *56:* 127–129.

COHN, W. E. The determination of hemoglobin in tissue extracts or other turbid solutions. *J. Biol. Chem.*, 1943, *148:* 219–223.

COOPER, F. W., JR., STEAD, E. A., JR., AND WARREN, J. V. The beneficial effect of intravenous infusions in acute pericardial tamponade. *Ann. Surg.*, 1944, *120:* 822–825.

COPE, O., AND RHINELANDER, F. W. The problem of burn shock complicated by pulmonary damage. *Ann. Surg.*, 1943, *117:* 915–928.

CORCORAN, A. C., AND PAGE, I. H. Post-traumatic renal injury. Summary of experimental observations. *Arch. Surg.*, 1945, *51:* 93–101.

CORCORAN, A. C., AND PAGE, I. H. Renal damage from Ferroheme pigments, myoglobin, hemoglobin, hematin. *Texas Reports Biol. & Med.*, 1945, *3:* 528–544.

CORCORAN, A. C., TAYLOR, R. D., AND PAGE, I. H. Immediate effects on renal function of the onset of shock due to partially occluding limb tourniquets. *Ann. Surg.*, 1943, *118:* 871–886.

COURNAND, A. Studies of the circulation in shock. *Proc. Rudolf Virchow Med. Soc.*, 1943, *2:* 151–155.

COURNAND, A., NOBLE, R. P., BREED, E. S., LAUSON, H. D., BALDWIN, E. DEF., PINCHOT, G. B., AND RICHARDS, D. W., JR. Chemical, clinical, and immunological studies on the products of human plasma fractionation. VIII. Clinical use of concentrated human serum albumin in shock, and comparison with whole blood and with rapid saline infusion. *J. Clin. Investigation*, 1944, *23:* 491–505.

COURNAND, A., RILEY, R. L., BRADLEY, S. E., BREED, E. S., NOBLE, R. P., LAUSON, H. D., GREGERSEN, M. I., AND RICHARDS, D. W., JR. Studies of the circulation in clinical shock. *Surgery*, 1943, *13:* 964–995.

DAVIDSON, C. S., LEWIS, J. H., TAGNON, H. J., ADAMS, M. A., AND TAYLOR, F. H. L. Medical shock: Abnormal biochemical changes in patients with severe, acute medical illnesses, with and without peripheral vascular failure. *New England J. Med.*, 1946, *234:* 279–288.

DETURK, W. E., AND GREIG, M. E. Studies on shock induced by hemorrhage. IX. The inhibition of amino acid oxidation in shock induced by hemorrhage. *J. Pharmacol. & Exper. Therap.*, 1945, *83:* 220–224.

DEXTER, L., FRANK, H. A., HAYNES, F. W., AND ALTSCHULE, M. D. Traumatic shock. VI. The effect of hemorrhagic shock on the concentration of renin and hypertensinogen in the plasma in unanesthetized dogs. *J. Clin. Investigation*, 1943, *22:* 847–852.

DEXTER, L., HAYNES, F. W., AND BRIDGES, W. C. The renal humoral pressor mechanism in man. I. Preparation and assay of human renin, human hypertensinogen, and hypertensin. *J. Clin. Investigation*, 1945, *24:* 62–68.

DEYRUP, I. J. Circulatory changes following the subcutaneous injection of histamine in dogs. *Am. J. Physiol.*, 1944, *142:* 158–172.

DUNCAN, G. W. Inorganic phosphorus content of the serum in shock. *Arch. Surg.*, 1943, *46:* 214–223.

DUNCAN, G. W. Venous pressure as an index of blood flow in the upper extremity. *Arch. Surg.*, 1944, *49:* 235–237.

DUNCAN, G. W., AND BLALOCK, A. The effects of the application of a tourniquet on the general response to gross trauma to an extremity. *Surgery*, 1943, *13:* 401–405.

DUNCAN, G. W., AND BLALOCK, A. Shock produced by crush injury: Effects of the administration of plasma and the local application of cold. *Arch. Surg.*, 1942, *45:* 183–194.

DUNCAN, G. W., AND BLALOCK, A. The uniform production of experimental shock by crush injury: Possible relationship to clinical crush syndrome. *Ann. Surg.*, 1942, *115:* 684–697.

DUNCAN, G. W., HYMAN, C., AND CHAMBERS, E. L. Determination of blood pressure in rats by direct observation of blood vessels. *J. Lab. & Clin. Med.*, 1943, *28:* 886–889.

DUNCAN, G. W., IRVIN, J. L., AND SARNOFF, S. J. Non-protein nitrogen and protein concentrations of serum and cerebrospinal fluid in shock. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 135–143.

DUNCAN, G. W., SARNOFF, S. J., AND RHODE, C. M. Studies on the effects of posture in shock and injury. *Ann. Surg.*, 1944, *120:* 24–33.

DUNPHY, J. E., AND GIBSON, J. G., II. The effect of infusions of bovine serum albumin in experimental shock. *Surgery*, 1943, *14:* 509–518.

EVANS, E. I. The significance of blood volume alterations in surgical patients. *South. M. J.*, 1945, *38:* 214–221.

EVANS, E. I. Studies on traumatic shock. III. Anesthesia in clinical shock. *J.A.M.A.*, 1944, *124:* 473–478.

EVANS, E. I., HOOVER, M. J., JAMES, G. W., AND ALM, T. Studies on traumatic shock. I. Blood volume changes in traumatic shock. *Ann. Surg.*, 1944, *119:* 64–85.

EVANS, E. I., JAMES, G. W., AND HOOVER, M. J. Studies on traumatic shock. II. The restoration of blood volume in traumatic shock. *Surgery*, 1944, *15:* 420–431.

EVANS, E. I., AND RAFAL, H. S. Studies on traumatic shock: V. The treatment of clinical shock with gelatin. *Ann. Surg.*, 1945, *121:* 478–494.

EVERSOLE, W. J., KLEINBERG, W., OVERMAN, R. R., REMINGTON, J. W., AND SWINGLE, W. W. The nervous factor in shock induced by muscle trauma in normal dogs. *Am. J. Physiol.*, 1944, *140:* 490–498.

FINE, J., FRANK, H. A., AND SELIGMAN, A. M. Traumatic shock incurable by volume replacement therapy. A summary of further studies including observations on the hemodynamics, intermediary metabolism, and therapeutics of shock. *Ann. Surg.*, 1945, *122:* 652–662.

FINE, J., FRANK, H. A., AND SELIGMAN, A. M. Traumatic shock. VIII. Studies in

the therapy and hemodynamics of tourniquet shock. *J. Clin. Investigation,* 1944, *23:* 731–741.

FINE, J., AND SELIGMAN, A. M. Traumatic shock. IV. A study of the problem of the "lost plasma" in hemorrhagic shock by the use of radioactive plasma protein. *J. Clin. Investigation,* 1943, *22:* 285–303.

FINE, J., AND SELIGMAN, A. M. Traumatic shock. VII. A study of the problem of the "lost plasma" in hemorrhagic, tourniquet, and burn shock by the use of radioactive iodo-plasma protein. *J. Clin. Investigation,* 1944, *23:* 720–730.

FINE, J., AND SELIGMAN, A. M. Traumatic shock. An experimental study including evidence against the capillary leakage hypothesis. *Ann. Surg.,* 1943, *118:* 238–255.

FINK, R. M., ENNS, T., KIMBALL, C. P., SILBERSTEIN, H. E., BALE, W. F., MADDEN, S. C., AND WHIPPLE, G. H. Plasma protein metabolism — normal and associated with shock. Observations using protein labeled by heavy nitrogen in lysine. *J. Exper. Med.,* 1944, *80:* 455–475.

FOX, C. L., JR. Oral sodium lactate in the treatment of burn shock. *J.A.M.A.,* 1944, *124:* 207–212.

FOX, C. L., JR., AND KESTON, A. S. The mechanism of shock from burns and trauma traced with radiosodium. *J. Clin. Investigation,* 1944, *23:* 935.

FRANK, H. A., ALTSCHULE, M. D., AND ZAMCHECK, N. Traumatic shock. IX. Pressor therapy: The effect of paredrine (p-hydroxy-a-methylphenylethylamine hydrobromide) on the circulation in hemorrhagic shock in dogs. *J. Clin. Investigation,* 1945, *24:* 54–61.

FRANK, H. A., AND FINE, J. Traumatic shock. V. A study of the effect of oxygen on hemorrhagic shock. *J. Clin. Investigation,* 1943, *22:* 305–314.

FRANK, H. A., SELIGMAN, A. M., AND FINE, J. Traumatic shock. X. The treatment of hemorrhagic shock irreversible to replacement of blood volume deficiency. *J. Clin. Investigation,* 1945, *24:* 435–444.

FRANK, H. A., SELIGMAN, A. M., AND FINE, J. Traumatic shock, XIII. The prevention of irreversibility in hemorrhagic shock by viviperfusion of the liver. *J. Clin. Investigation,* 1945, *25:* 22–29.

FRANK, H. A., SELIGMAN, A. M., AND FINE, J. Treatment of uremia after acute renal failure by peritoneal irrigation. *J.A.M.A.,* 1946, *130:* 703–705.

FURCHGOTT, R. F., AND SHORR, E. Sources of energy for intestinal smooth muscle contraction. *Proc. Soc. Exper. Biol. & Med.,* 1946, *61:* 280–286.

GELLHORN, A., MERRELL, M., AND RANKIN, R. M. The rate of transcapillary exchange of sodium in normal and shocked dogs. *Am. J. Physiol.,* 1944, *142:* 407–427.

GIBSON, J. G., II, SELIGMAN, A. M., AND PEACOCK, W. C. The capillary circulation of the normal dog. *J. Clin. Investigation,* 1944, *23:* 940.

GOLDBERG, M., AND FINE, J. Traumatic shock. XI. Intestinal absorption in hemorrhagic shock. *J. Clin. Investigation,* 1945, *24:* 445–450.

GOVIER, W. M. Studies on shock induced by hemorrhage. III. The correlation of plasma thiamin content with resistance to shock in dogs. *J. Pharmacol. & Exper. Therap.,* 1943, *77:* 40–49.

GOVIER, W. M., AND GREIG, M. E. Studies on shock induced by hemorrhage. V. The effect of cellular damage on the in vitro hydrolysis and synthesis of cocarboxylase by liver tissue. *J. Pharmacol. & Exper. Therap.,* 1943, *79:* 240–245.

GREGERSEN, M. I. Practical method for the determination of blood volume with dye T–1824; survey of present basis of dye-method and its clinical application. *J. Lab. & Clin. Med.*, 1944, *29:* 1266–1286. *Privately printed by Josiah Macy Jr. Foundation,* 1944.

GREGERSEN, M. I. Traumatic shock. *Bull. New York Acad. Med.*, 1943, *19:* 666.

GREGERSEN, M. I., BOYDEN, A. A., AND ALLISON, J. B. Comparison of plasma volume measured with the blue dye T–1824 and with beef albumin, beef globulin, and the pneumococcus polysaccharide SIII. *Federation Proc.*, 1945, *4:* 27.

GREIG, M. E. Studies on shock induced by hemorrhage. VII. The destruction of cozymase and alloxazine adenine dinucleotide in tissues during shock. *J. Pharmacol. & Exper. Therap.*, 1944, *81:* 164–173.

GREIG, M. E. Studies on shock induced by hemorrhage. VIII. The inactivation of the apoenzyme of amino acid oxidase and lactic dehydrogenase in anoxia. *J. Pharmacol. & Exper. Therap.*, 1944, *81:* 240–247.

GREIG, M. E., AND GOVIER, W. M. Studies on shock induced by hemorrhage. IV. The dephosphorylation of cocarboxylase in tissues during shock and anoxia. *J. Pharmacol. & Exper. Therap.*, 1943, *79:* 169–175.

GREIG, M. E., AND GOVIER, W. M. Studies on shock induced by hemorrhage. VI. Cocarboxylase phosphatase in dog serum. *J. Pharmacol. & Exper. Therap.*, 1943, *79:* 246–249.

HAMILTON, A. S., AND PARKINS, W. M. An evaluation of the effectiveness of globin, albumin, hemoglobin and oxypolygelatin in the treatment of hemorrhagic shock. *Am. J. M. Sc.*, 1945, *210:* 808–809.

HARKINS, H. N., AND LONG, C. N. H. Metabolic changes in shock after burns. *Am. J. Physiol.*, 1945, *144:* 661–668.

JANEWAY, C. A. Clinical use of products of human plasma fractionation. I. Albumin in shock and hypoproteinemia. II. Gamma-globulin in measles. *J.A.M.A.*, 1944, *126:* 674–680.

KATZIN, L. I., RICCA, R. A., AND WARREN, S. L. Effect of environmental temperature and anesthesia on the survival of tourniquet shock in rabbits. *J. Clin. Investigation*, 1945, *24:* 149–151.

KATZIN, L. I., AND WARREN, S. L. Thiamine-deficient diet in tourniquet shock in rats. *J. Clin. Investigation*, 1945, *24:* 152–153.

KETY, S. S., NATHANSON, I. T., NUTT, A. L., POPE, A., ZAMECNIK, P. C., AUB, J. C., AND BRUES, A. M. The toxic factors in experimental traumatic shock. III. Shock accompanying muscle ischemia and loss of vascular fluid. *J. Clin. Investigation*, 1945, *24:* 839–844.

KETY, S. S., AND POPE, A. The cardiovascular system in traumatic shock. *Am. Heart J.*, 1944, *27:* 601–609.

KLEINBERG, W., REMINGTON, J. W., DRILL, V. A., AND SWINGLE, W. W. The nervous factor in the circulatory failure induced in adrenalectomized dogs by intestinal stripping and a single stage bilateral adrenalectomy. *Am. J. Physiol.*, 1942, *137:* 362–370.

KLEINBERG, W., REMINGTON, J. W., EVERSOLE, W. J., OVERMAN, R. R., AND SWINGLE, W. W. The effectiveness of plasma, gelatin, and saline transfusions in preventing shock induced by leg muscle trauma and tourniquets. *Am. J. Physiol.*, 1943, *140:* 197–204.

KLINE, D. L. The effect of hemorrhage upon the amino nitrogen concentration of the blood. *Federation Proc.*, 1945, *4*: 40–41.

LAMSON, P. D., AND DeTURK, W. E. Studies on shock induced by hemorrhage. XI. A method for the accurate control of blood pressure. *J. Pharmacol. & Exper. Therap.*, 1945, *83*: 250–252.

LAMSON, P. D., AND GREIG, M. E. Certain biochemical aspects of shock from hemorrhage. *Tr. Am. Physicians*, 1944, *58*: 182–188.

LAMSON, P. D., ROBBINS, B. H., AND GREIG, M. E. Studies on shock induced by hemorrhage. X. Hemoglobin solutions as blood substitutes. *J. Pharmacol. & Exper. Therap.*, 1945, *83*: 225–234.

LAUSON, H. D., BRADLEY, S. E., AND COURNAND, A. The renal circulation in shock. *J. Clin. Investigation*, 1944, *23*: 381–402.

LEPAGE, G. A. Biological energy transformations during shock as shown by tissue analyses. *Am. J. Physiol.*, 1946, *146*: 267–281.

LEVINE, R., HUDDLESTUN, B., PERSKY, H., AND SOSKIN, S. The successful treatment of so-called "irreversible" shock by whole blood supplemented with sodium bicarbonate and glucose. *Am. J. Physiol.*, 1944, *141*: 209–215. *Federation Proc.*, 1944, *3*: 28.

LONGMIRE, W. P., JR., DUNCAN, G. W., AND BLALOCK, A. The use of venous tourniquets as an aid to the diagnosis of incipient traumatic shock. *Surg., Gynec. & Obst.*, 1944, *79*: 434–437.

MAHONEY, E. B., HOWLAND, J. W., AND YACKEL, K. The role of infection in shock produced by muscle injury. *Surgery*, 1945, *17*: 805–815.

McSHAN, W. H., POTTER, V. R., GOLDMAN, A., SHIPLEY, E. G., AND MEYER, R. K. Biological energy transformations during shock as shown by blood chemistry. *Am. J. Physiol.*, 1945, *145*: 93–106.

MERRILL, A. J., WARREN, J. V., STEAD, E. A., JR., AND BRANNON, E. S. The circulation in penetrating wounds of the chest: A study by the method of right heart catheterization. *Am. Heart J.*, 1946, *31*: 413–417.

MEYER, R. K., AND SHIPLEY, E. G. The production of shock in rats by the drum method. *Proc. Soc. Exper. Biol. & Med.*, 1945, *60*: 334–337.

MYLON, E., CASHMAN, C. W., JR., AND WINTERNITZ, M. C. The relation of adrenalin and of the carotid sinus to the hyperglycemia of shock. *Am. J. Physiol.*, 1944, *142*: 638–647.

MYLON, E., CASHMAN, C. W., JR., AND WINTERNITZ, M. C. Studies on mechanisms involved in shock and its therapy. *Am. J. Physiol.*, 1944, *142*: 299–309.

MYLON, E., AND WINTERNITZ, M. C. Factors concerned with the induction of tourniquet shock. *Am. J. Physiol.*, 1945, *144*: 494–504.

NATHANSON, I. T., NUTT, A. L., POPE, A., ZAMECNIK, P. C., AUB, J. C., BRUES, A. M., AND KETY, S. S. The toxic factors in experimental traumatic shock. I. Physiologic effects of muscle ligation in the dog. *J. Clin. Investigation*, 1945, *24*: 829–834.

NICKERSON, J. L. Local fluid loss in trauma. *Am. J. Physiol.*, 1945, *144*: 429–436.

NICKERSON, J. L. The low frequency critically-damped ballistocardiograph. *Federation Proc.*, 1945, *4*: 201–206.

NICKERSON, J. L. A portable decade photometer for the determination of blood volume with dye T–1824. *Rev. Scient. Instruments*, 1944, *15*: 69–72.

NICKERSON, J. L., AND CURTIS, H. J. The design of the ballistocardiograph. *Am. J. Physiol.,* 1944, *142:* 1–11.

NICKERSON, J. L., KING, B. G., AND CURTIS, H. J. A small recording spirometer. *Rev. Scient. Instruments,* 1944, *15:* 12–13.

OVERMAN, R. R., AND WANG, S. C. The apparent nervous factor in experimental shock. *Federation Proc.,* 1945, *4:* 54–55.

PAINTER, E. E., WANG, S. C., AND OVERMAN, R. R. The fluorescein circulation time in experimental traumatic shock. *Federation Proc.,* 1945, *4:* 55.

PHEMISTER, D. B. Role of the nervous system in shock. *Ann. Surg.,* 1943, *118:* 256–268.

PHEMISTER, D. B., EICHELBERGER, L., AND LAESTAR, C. H. Early effects on dogs of section of the eighth cervical segment of the spinal cord and their bearing on shock. *Arch. Surg.,* 1945, *51:* 32–41.

PHEMISTER, D. B., LAESTAR, C. H., EICHELBERGER, L., AND SCHACHTER, R. J. Afferent vasodepressor nerve impulses as a cause of shock: Tested experimentally by aortic-depressor nerve stimulation. *Ann. Surg.,* 1944, *119:* 26–63.

PHEMISTER, D. B., AND SCHACHTER, R. J. Neurogenic shock. I. The effects of prolonged lowering of blood pressure by continuous stimulation of the carotid sinus in dogs. *Ann. Surg.,* 1942, *116:* 610–622.

POLDERMAN, H., MCCARRELL, J. D., AND BEECHER, H. K. Effect of anesthesia on lymph flow (local procaine, ether, pentobarbital sodium). *J. Pharmacol. & Exper. Therap.,* 1943, *78:* 400–406.

POPE, A., ZAMECNIK, P. C., AUB, J. C., BRUES, A. M., DUBOS, R. J., NATHANSON, I. T., AND NUTT, A. L. The toxic factors in experimental traumatic shock. VI. The toxic influence of the bacterial flora, particularly Clostridium welchii, in exudates of ischemic muscle. *J. Clin. Investigation,* 1945, *24:* 856–863.

POTTER, V. R. Coupling between phosphorylation and oxidation of the 4-carbon acids in rat kidney homogenates. *Arch. Biochem.,* 1945, *6:* 439–453.

REMINGTON, J. W. Effect of adrenal cortical extract and certain steroid hormones on the response of adrenalectomized rats to intraperitoneal injections of an isotonic glucose solution. *Endocrinology,* 1943, *32:* 129–134.

REMINGTON, J. W., DRILL, V. A., KLEINBERG, W., AND SWINGLE, W. W. Changes in blood chemistry associated with circulatory failure in the adrenalectomized dog. *Endocrinology,* 1942, *30:* 692–701.

RHOADS, J. E., WOLFF, W. A., SALTONSTALL, H., AND LEE, W. E. Further experiences with adrenal cortical extract in the treatment of burn shock. *Ann. Surg.,* 1943, *118:* 982–987.

RICCA, R. A., FINK, K., KATZIN, L. I., AND WARREN, S. L. Effect of environmental temperature on experimental traumatic shock in dogs. *J. Clin. Investigation,* 1945, *24:* 127–139.

RICCA, R. A., FINK, K., STEADMAN, L. T., AND WARREN, S. L. The distribution of body fluids of dogs in traumatic shock. *J. Clin. Investigation,* 1945, *24:* 140–145.

RICCA, R. A., FINK, K., AND WARREN, S. L. The effect of sulfadiazine, antitoxins, globulins, and dog plasma on dogs in traumatic shock under sodium pentobarbital anesthesia. *J. Clin. Investigation,* 1945, *24:* 146–148.

RICHARDS, D. W., JR. The circulation in traumatic shock in man. Harvey Lecture Series, 1943–1944, *39:* 217–253. *Bull. New York Acad. Med.,* 1944, *20:* 363–393.

RICHARDS, D. W., JR., AND COURNAND, A. Circulation in shock: Mechanical and vasomotor factors. *Tr. A. Am. Physicians,* 1944, *58:* 111.

ROOT, W. S., ROUGHTON, F. J. W., AND GREGERSEN, M. I. Simultaneous measurement of blood volume with dye (T–1824) and with carbon monoxide (improved method). *Federation Proc.,* 1945, *4:* 60.

SAYERS, G., SAYERS, M. A., LIANG, TSAN-YING, AND LONG, C. N. H. The cholesterol and ascorbic acid content of the adrenal, liver, brain, and plasma following hemorrhage. *Endocrinology,* 1945, *37:* 96–110.

SAYERS, M. A., SAYERS, G., ENGEL, M. G., ENGEL, F. L., AND LONG, C. N. H. Elevation of plasma amino nitrogen as an index of the gravity of hemorrhagic shock. *Proc. Soc. Exper. Biol. & Med.,* 1945, *60:* 20–22.

SCHOLZ, D. E., SCHULTZ, J. H., PLEUNE, F. G., FINK, K., STEADMAN, L. T., AND WARREN, S. L. Study of the body temperature and water content in shock produced by the continuous intravenous injection of adrenalin, with and without anesthesia. *J. Clin. Investigation,* 1945, *24:* 154–159.

SELIGMAN, A. M. Traumatic shock. III. A modified electroscope especially suited for measuring substances with low energy radiation. *J. Clin. Investigation,* 1943, *22:* 281–284.

SELIGMAN, A. M., AND FINE, J. Traumatic shock. I. The production of radioactive plasma protein from amino acids containing radioactive sulfur. *J. Clin. Investigation,* 1943, *22:* 265–273.

SELIGMAN, A. M., FRANK, H. A., AND FINE, J. Traumatic shock. XII. Hemodynamic effects of alterations of blood viscosity in normal dogs and in dogs in shock. *J. Clin. Investigation,* 1946, *25:* 1–21.

SELIGMAN, A. M., FRANK, H. A., AND FINE, J. Treatment of experimental uremia by means of peritoneal irrigation. *J. Clin. Investigation,* 1946, *25:* 211–219.

SELIGMAN, A. M., RUTENBURG, A. M., AND BANKS, H. Traumatic shock. II. The preparation of cystine, methionine, and homocystine containing radioactive sulfur. *J. Clin. Investigation,* 1943, *22:* 275–279.

SHIPLEY, E. G., MEYER, R. K., AND McSHAN, W. H. Shock produced by the application of tourniquets to the hind limbs of rats. *Proc. Soc. Exper. Biol. & Med.,* 1945, *60:* 340–344.

SHORR, E., ZWEIFACH, B. W., AND FURCHGOTT, R. F. On the occurrence, sites and modes of origin and destruction, of principles affecting the compensatory vascular mechanisms in experimental shock. *Science,* 1945, *102:* 489–498.

STEAD, E. A., JR., AND WARREN, J. V. The effect of the injection of histamine into the brachial artery on the permeability of the capillaries of the forearm and hand. *J. Clin. Investigation,* 1944, *23:* 279–281.

STEAD, E. A., JR., AND WARREN, J. V. Orientation to the mechanisms of clinical shock. *Arch. Surg.,* 1945, *50:* 1–5.

STEAD, E. A., JR., AND WARREN, J. V. The protein content of the extracellular fluid in normal subjects after venous congestion and in patients with cardiac failure, anoxemia, and fever. *J. Clin. Investigation,* 1944, *23:* 283–287.

SWINGLE, W. W., KLEINBERG, W., REMINGTON, J. W., EVERSOLE, W. J., AND OVERMAN, R. R. Experimental analysis of the nervous factor in shock induced by muscle trauma in normal dogs. *Am. J. Physiol.,* 1944, *141:* 54–63.

SWINGLE, W. W., OVERMAN, R. R., REMINGTON, J. W., KLEINBERG, W., AND EVERSOLE, W. J. Ineffectiveness of adrenal cortex preparations in the treatment

of experimental shock in nonadrenalectomized dogs. *Am. J. Physiol.*, 1943, *139:* 481–489.

SWINGLE, W. W., REMINGTON, J. W., DRILL, V. A., AND KLEINBERG, W. Differences among adrenal steroids with respect to their efficacy in protecting the adrenalectomized dog against circulatory failure. *Am. J. Physiol.*, 1942, *136:* 567–576.

SWINGLE, W. W., REMINGTON, J. W., KLEINBERG, W., DRILL, V. A., AND EVERSOLE, W. J. An experimental study of the tourniquet as a method for inducing circulatory failure in the dog. *Am. J. Physiol.*, 1942, *138:* 156–165.

TAGNON, H. J., LEVENSON, S. M., DAVIDSON, C. S., AND TAYLOR, F. H. L. The occurrence of fibrinolysis in shock, with observations on the prothrombin time and the plasma fibrinogen during hemorrhagic shock. *Am. J. M. Sc.*, 1946, *211:* 88–96.

THOMSON, J. E. M., HELWIG, F. C., AND SIRE, E. Sympathetic block in the treatment of local shock; an experimental study. *J. Bone & Joint Surg.*, 1944, *26:* 189–196.

VAN SLYKE, D. D., PHILLIPS, R. A., HAMILTON, P. B., ARCHIBALD, R. M., DOLE, V. P., AND EMERSON, K., JR. Effect of shock on the kidney. *Tr. A. Am. Physicians*, 1944, *58:* 119–127.

WALCOTT, W. W. Blood volume in experimental hemorrhagic shock. *Am. J. Physiol.*, 1945, *143:* 247–253.

WALCOTT, W. W. A standard method of producing shock in dogs by a single massive hemorrhage. *Proc. Soc., Exper. Biol. & Med.*, 1944, *55:* 272.

WALCOTT, W. W. Standardization of experimental hemorrhagic shock. *Am. J. Physiol.*, 1945, *143:* 254–261.

WANG, S. C., OVERMAN, R. R., ROOT, W. S., AND GREGERSEN, M. I. Relation of the blood volume reduction to the mortality rates in experimental traumatic and hemorrhagic shock. *Federation Proc.*, 1945, *4:* 75.

WARREN, J. V., BRANNON, E. S., AND MERRILL, A. J. A method of obtaining renal venous blood in unanesthetized persons with observations on the extraction of oxygen and sodium para-amino hippurate. *Science,* 1944, *100:* 108–110.

WARREN, J. V., BRANNON, E. S., STEAD, E. A., JR., AND MERRILL, A. J. The effect of venesection and the pooling of blood in the extremities on the atrial pressure and cardiac output in normal subjects with observations on acute circulatory collapse in three instances. *J. Clin. Investigation.* 1945, *24:* 337–344.

WARREN, J. V., BRANNON, E. S., STEAD, E. A., JR., AND MERRILL, A. J. Pericardial tamponade from stab wound of the heart and pericardial effusion or empyema: A study utilizing the method of right heart catheterization. *Am. Heart J.*, 1946, *31:* 418–425.

WARREN, J. V., MERRILL, A. J., AND STEAD, E. A., JR. The role of the extracellular fluid in the maintenance of a normal plasma volume. *J. Clin. Investigation,* 1943, *22:* 635–641.

WARREN, J. V., STEAD, E. A., JR., MERRILL, A. J., AND BRANNON, E. S. Chemical, clinical, and immunological studies on the products of human plasma fractionation. IX. The treatment of shock with concentrated human serum albumin: A preliminary report. *J. Clin. Investigation,* 1944, *23:* 506–509.

WILHELMI, A. E., RUSSELL, J. A., ENGEL, F. L., AND LONG, C. N. H. The effects

of hepatic anoxia on the respiration of liver slices in vitro. *Am. J. Physiol.*, 1945, *144*: 669–673.

WILHELMI, A. E., RUSSELL, J. A., ENGEL, M. G., AND LONG, C. N. H. Some aspects of the nitrogen metabolism of liver tissue from rats in hemorrhagic shock. *Am. J. Physiol.*, 1945, *144*: 674–682.

WILHELMI, A. E., RUSSELL, J. A., LONG, C. N. H., AND ENGEL, M. G. The effects of anoxia and of hemorrhage upon the metabolism of the cerebral cortex of the rat. *Am. J. Physiol.*, 1945, *144*: 683–692.

ZAMECNIK, P. C., AUB, J. C., BRUES, A. M., KETY, S. S., NATHANSON, I. T., NUTT, A. L., AND POPE, A. The toxic factors in experimental traumatic shock. V. Chemical and enzymatic properties of muscle exudate. *J. Clin. Investigation*, 1945, *24*: 850–855.

ZWEIFACH, B. W., LEE, R. E., HYMAN, C., AND CHAMBERS, R. Omental circulation in morphinized dogs subjected to graded hemorrhage. *Ann. Surg.*, 1944, *120*: 232–250.

ZWEIFACH, B. W., LOWENSTEIN, B. E., AND CHAMBERS, R. Responses of blood capillaries to acute hemorrhage in the rat. *Am. J. Physiol.*, 1944, *142*: 80–93.

Blood, Blood Derivatives and Blood Substitutes

BAILEY, O. T., AND HAWN, C. Z. The effect of crystallized bovine serum albumin on the tissues of normal animals; morphologic changes in normal rabbits induced by intravenous injection of crystallized bovine serum albumin. *Am. J. Path.*, 1943, *19*: 267–291.

BALLOU, G. A., BOYER, P. D., AND LUCK, J. M. The electrophoretic mobility of human serum albumin as affected by lower fatty acid salts. *J. Biol. Chem.*, 1945, *159*: 111–116.

BALLOU, G. A., BOYER, P. D., LUCK, J. M., AND LUM, F. G. Chemical, clinical, and immunological studies on the products of human plasma fractionation. V. The influence of non-polar anions on the thermal stability of serum albumin. *J. Clin. Investigation*, 1944, *23*: 454–457.

BALLOU, G. A., BOYER, P. D., LUCK, J. M., AND LUM, F. G. The heat coagulation of human serum albumin. *J. Biol. Chem.*, 1944, *153*: 589–605.

BASSETT, D. L. Standard human serum albumin stabilized with sodium phenylacetate and sodium mandelate. Toxicity studies. *Am. J. Physiol.*, 1945, *143*: 272–276.

BENDICH, A., KABAT, E. A., AND BEZER, A. E. Immunochemical studies on blood groups. III. Properties of purified blood group A substances from individual hog stomach linings. *J. Exper. Med.*, 1946, *83*: 485–497.

BERING, E. A., JR. Clinical uses of products made from human fibrinogen and thrombin. *Bull. U. S. Army Med. Dept.*, 1944, *78*: 53–56.

BOYD, W. C. A nomogram for acetate buffers. *J. Am. Chem. Soc.*, 1945, *67*: 1035.

BOYER, P. D., BALLOU, G. A., AND LUCK, J. M. The combination of fatty acids and related compounds with serum albumin. II. Stabilization against urea and guanidine denaturation. *J. Biol. Chem.*, 1946, *162*: 199–208.

BOYER, P. D., LUM, F. G., BALLOU, G. A., LUCK, J. M., AND RICE, R. G. The combination of fatty acids and related compounds with serum albumin. I. Stabilization against heat denaturation. *J. Biol. Chem.*, 1946, *162*: 181–198.

BRAND, E., KASSELL, B., AND SAIDEL, L. J. Chemical, clinical, and immunological studies on the products of human plasma fractionation. III. Amino acid composition of plasma proteins. *J. Clin. Investigation,* 1944, *23:* 437–444.

BRAND, E., RYAN, F. J., AND DISKANT, E. M. Leucine content of proteins and foodstuffs. *J. Am. Chem. Soc.,* 1945, *67:* 1532–1534.

BRAND, E., SAIDEL, L. J., GOLDWATER, W. H., KASSELL, B., AND RYAN, F. J. The empirical formula of beta-lactoglobulin. *J. Am. Chem. Soc.,* 1945, *67:* 1524–1532.

CHASE, A. M., LORENZ, P. B., PARPART, A. K., AND GREGG, J. R. A method for simultaneous determination of methemoglobin-oxyhemoglobin ratio and per cent hemolysis in stored blood. *J. Lab. & Clin. Med.,* 1944, *29:* 99–105.

COHN, E. J. Blood and blood derivatives. *Am. Scientist,* 1945, *33:* 61–83.

COHN, E. J. Blood, blood derivatives, and blood substitutes. *Proc. Am. Philosophical Soc.,* 1944, *88:* 159–173.

COHN, E. J. Blood proteins and their therapeutic value. *Science,* 1945, *101:* 51–56.

COHN, E. J. The plasma proteins: Their properties and functions. *Tr. & Studies College Physicians,* Philadelphia, 1942, *10:* 149–162.

COHN, E. J., ONCLEY, J. L., STRONG, L. E., HUGHES, W. L., JR., AND ARMSTRONG, S. H., JR. Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. *J. Clin. Investigation,* 1944, *23:* 417–432.

COOPER, G. R. Electrophoretic analysis of mixtures of protein. *J. Biol. Chem.,* 1945, *158:* 727–728.

DIAMOND, L. K. The practical importance of the Rh blood type and a project for the collection and preparation of Rh typing serum. *Connecticut State M. J.,* 1944, *8:* 505–507.

DIAMOND, L. K. Rh typing serum. *New England J. Med.,* 1944, *230:* Letter to the Editor, June 29.

DIAMOND, L. K., AND ABELSON, N. M. The demonstration of anti-Rh agglutinins — an accurate and rapid slide test. *J. Lab. & Clin. Med.,* 1945, *30:* 204–212.

DIAMOND, L. K., AND ABELSON, N. M. The detection of Rh sensitization: Evaluation of tests for Rh antibodies. *J. Lab. & Clin. Med.,* 1945, *30:* 668–674.

DIAMOND, L. K., AND ABELSON, N. M. The importance of Rh inhibitor substance in anti-Rh serums. *J. Clin. Investigation,* 1945, *24:* 122–126.

DIGGS, L. W. Disputed points concerning technical procedures used in transfusions by the citrate method. *Memphis M. J.,* 1942, *17:* 186–190.

DIGGS, L. W. The efficiency of various types of equipment used in the collection of blood for transfusion. I. Needles and adapters. *Am. J. Clin. Path.,* 1942, *12:* 518–522.

DIGGS, L. W. The milk bottle as a blood transfusion flask. *Am. J. Clin. Path.,* 1943, *13:* 101–107.

DIGGS, L. W., AND SMITH, J. M. A mechanical shaker for transfusion flasks using a standard electric motor. *Am. J. Clin. Path.,* 1943, *13:* 67–69.

EDSALL, J. T., FERRY, R. M., AND ARMSTRONG, S. H., JR. Chemical, clinical and immunological studies on the products of human plasma fractionation. XV. The proteins concerned in the blood coagulation mechanism. *J. Clin. Investigation,* 1944, *23:* 557–565.

ENDERS, J. F. Chemical, clinical, and immunological studies on the products of

human plasma fractionation. X. The concentrations of certain antibodies in globulin fractions derived from human blood plasma. *J. Clin. Investigation,* 1944, *23:* 510–530.

FERRY, J. D., AND MORRISON, P. R. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XVI. Fibrin clots, fibrin films, and fibrinogen plastics. *J. Clin. Investigation,* 1944, *23:* 566–572.

FOSTER, J. F., SCHEINBERG, H., AND EDSALL, J. T. Molecular length of fibrinogen and zein as deduced from double refraction of flow. *Federation Proc.,* 1944, *3:* 57.

HEYL, J. T., GIBSON, J. G., II, AND JANEWAY, C. A. Studies on the plasma proteins. V. The effect of concentrated solutions of human and bovine serum albumin on blood volume after acute blood loss in man. *J. Clin. Investigation,* 1943, *22:* 763–773.

HOLMES, F. E. A storage vessel for blood. *J. Lab. & Clin. Med.,* 1942, *28:* 121–122.

JANEWAY, C. A., GIBSON, S. T., WOODRUFF, L. M., HEYL, J. T., BAILEY, O. T., AND NEWHOUSER, L. R. Chemical, clinical, and immunological studies on the products of human plasma fractionation. VII. Concentrated human serum albumin. *J. Clin. Investigation,* 1944, *23:* 465–490.

JENKINS, H. P., SCHAFER, P. W., AND OWENS, F. M., JR. Guide to replacement therapy for loss of blood or plasma. *Arch. Surg.,* 1943, *47:* 1–3.

KABAT, E. A., BENDICH, A., AND BEZER, A. E. Immunochemical studies on blood groups. II. Properties of the blood group A substance from pools of hog stomachs and of specific precipitates composed of "A" substance and homologous human antibody. *J. Exper. Med.,* 1946, *83:* 477–484.

LANNI, F., FEIGEN, G. A., AND LeROSEN, A. L. The determination of gelatin in the presence of plasma proteins. *Arch. Biochem.,* 1945, *8:* 251–257.

LOWELL, A., COLCHER, H., KENDALL, F. E., PATEK, A. J., JR., AND SEEGAL, D. A comparison of the effects of high and low viscosity gelatins after their intravenous injection in man. *J. Clin. Investigation,* 1946, *25:* 226–236.

LOZNER, E. L., TAYLOR, F. H. L., LEMISH, S., SNYDER, R., AND NEWHOUSER, L. R. Preservation of normal human plasma in the liquid state. III. Studies on chemical and physicochemical changes during the second year of storage. *J. Clin. Investigation,* 1944, *23:* 357–360.

MORRISON, P. R., AND SINGER, M. Chemical, clinical, and immunological studies on the products of human plasma fractionation, XVII. A note on the absorption rates of fibrin films in tissue. *J. Clin. Investigation,* 1944, *23:* 573–575.

PARPART, A. K., AND BALLENTINE, R. Hematocrit determination of relative cell volume. *Science,* 1943, *98:* 545.

PHILLIPS, R. A. A method for the determination of the blue dye T–1824 in plasma. *J. Exper. Med.,* 1943, *77:* 421–434.

PHILLIPS, R. A., VAN SLYKE, D. D., DOLE, V. P., EMERSON, K., JR., HAMILTON, P. B., AND ARCHIBALD, R. M. Copper sulfate method for measuring specific gravities of whole blood and plasma. *Bull. U. S. Army M. Dept.,* 1943, *No. 71:* 66–83.

Copper sulfate method for measuring specific gravities of whole blood and plasma with line charts for calculating plasma proteins, hemoglobin and hematocrit from plasma and whole blood gravities. *Privately printed by*

Josiah Macy, Jr. Foundation, 1945, February. Abstract of this paper in *Bull. Hyg.,* 1944, *19:* 140.

PILLEMER, L., ONCLEY, J. L., MELIN, M., ELLIOTT, J., AND HUTCHINSON, M. C. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XIII. The separation and concentration of isohemagglutinins from group-specific human plasma. *J. Clin. Investigation,* 1944, *23:* 550–553.

RICE, R. G., BALLOU, G. A., BOYER, P. D., LUCK, J. M., AND LUM, F. G. The papain digestion of native, denatured, and "stabilized" human serum albumin. *J. Biol. Chem.,* 1945, *158:* 609–617.

RYAN, F. J., AND BRAND, E. A method for the determination of leucine in protein hydrolysates and in foodstuffs by the use of a Neuro-spora mutant. *J. Biol. Chem.,* 1944, *154:* 161–175.

SCATCHARD, G., BATCHELDER, A. C., AND BROWN, A. Chemical, clinical, and immunological studies on the products of human plasma fractionation. VI. The osmotic pressure of plasma and of serum albumin. *J. Clin. Investigation,* 1944, *23:* 458–464.

SCATCHARD, G., GIBSON, S. T., WOODRUFF, L. M., BATCHELDER, A. C., AND BROWN, A. Chemical, clinical, and immunological studies on the products of human plasma fractionation. IV. A study of the thermal stability of human serum albumin. *J. Clin. Investigation,* 1944, *23:* 445–453.

SCATCHARD, G., ONCLEY, J. L., WILLIAMS, J. W., AND BROWN, A. Size distribution in gelatin solutions. Preliminary report. *J. Am. Chem. Soc.,* 1944, *66:* 1980–1981.

STATE, D., AND WANGENSTEEN, O. H. Procaine intravenously in treatment of delayed serum sickness. *J.A.M.A.,* 1946, *130:* 990–995.

STRUMIA, M. M. Filter-drip unit for dispensing blood and plasma. *Am. J. Clin. Path.,* 1944, *14:* 75–80.

STRUMIA, M. M. Preservation of prothrombin in dried plasma. *J.A.M.A.,* 1942, *119:* 710.

STRUMIA, M. M., BLAKE, A. D., REIDER, H. C., AND CHORNOCK, F. W. The use of a "modified globin" from human erythrocytes in hypoproteinemias. *Am. J. M. Sc.,* 1946, *211:* 51–61.

STRUMIA, M. M., CHORNOCK, F. W., BLAKE, A. D., AND KARR, W. G. The use of a "modified globin" from human erythrocytes as a plasma substitute: Preliminary report. *Am. J. M. Sc.,* 1945, *209:* 436–442.

STRUMIA, M. M., AND MCGRAW, J. J. A method and apparatus for shell freezing and rapid drying of plasma and other products from the frozen state by low temperature water vapor condensation in vacuo. *J. Lab. & Clin. Med.,* 1943, *28:* 1140–1155.

STRUMIA, M. M., MCGRAW, J. J., AND BLAKE, A. Reactions to parenteral fluid administration. *Ann. Int. Med.,* 1943, *19:* 718–734.

TAYLOR, F. H. L., LOZNER, E. L., DAVIDSON, C. S., TAGNON, H. J., AND NEWHOUSER, L. R. Preservation of normal human plasma in the liquid state. II. Comparative in vitro studies on the physiologic activity of labile constituents of liquid and frozen plasma. *J. Clin. Investigation,* 1944, *23:* 351–356.

WIENER, A. S., SOBLE, R., AND POLIVKA, H. Simple method of preparing potent blood grouping sera. *Proc. Soc. Exper. Biol. & Med.,* 1945, *58:* 310–312.

WILLIAMS, J. W., PETERMANN, M. L., COLOVOS, G. C., GOODLOE, M. B., ONCLEY, J. L., AND ARMSTRONG, S. H. JR. Chemical, clinical, and immunological studies on the products of human plasma fractionation. II. Electrophoretic and ultra-centrifugal studies of solutions of human serum albumin and immune serum globulins. *J. Clin. Investigation*, 1944, *23:* 433–436.

Nutrition

(See MEDICINE, Convalescence, for nutrition in convalescence.)
(See SURGERY, Wounds and Burns, for nutrition in burns.)
(See PHYSIOLOGY, Acclimatization, for nutrition and acclimatization.)

ANDERSON, M., MICKELSEN, O., AND KEYS, A. Diets and food items for the experimental production of thiamin deficiency in man. *J. Am. Dietet. A.,* 1946, *22:* 1–7.

BESSEY, O. A., AND LOWRY, O. H. Factors influencing the riboflavin content of the cornea. *J. Biol. Chem.,* 1944, *155:* 635–643.

BROZEK, J. Changes in specific gravity and body fat of young men under conditions of experimental semi-starvation. *Federation Proc.,* 1946, *5:* 13.

BROZEK, J., GUETZKOW, H., AND KEYS, A. A study of personality of normal young men maintained on restricted intakes of vitamins of the B complex. *Psychosom. Med.,* 1946, *8:* 98–109.

CARL, B. C., WATTS, B. M., AND MORGAN, A. F. Dehydration of meat scrapples. *Food Research,* 1944, *9:* 319–327.

CASTER, W. O., CONDIFF, H., MICKELSEN, O., AND KEYS, A. The excretion of pyrimidine and thiamine by man in different nutritional states. *Federation Proc.,* 1945, *4:* 85.

CASTER, W. O., MICKELSEN, O., AND KEYS, A. Relation between urinary excretion of thiamine and pyramin (the pyrimidine-like component of thiamine). *Federation Proc.,* 1946, *5:* 128.

DARLING, R. C., JOHNSON, R. E., PITTS, G. C., CONSOLAZIO, F. C., AND ROBINSON, P. F. Effects of variations in dietary protein on the physical well being of men doing manual work. *J. Nutrition,* 1944, *28:* 273–281.

FARMER, C. J. Some aspects of vitamin C metabolism. *Federation Proc.,* 1944, *3:* 179–188.

FENTON, F., ALBURY, M., VISNYEI, K., THOMPSON, J. F., AND GLEIM, E. Effect of quantity preparation procedures on vitamin retention and palatability of dehydrated potato shreds. *J. Am. Dietet.,* 1945, *21:* 518–521.

FENTON, F., GLEIM, E., ALBURY, M., VISNYEI, K., AND MCCARTNEY, J. R. Effect of quantity preparation procedures on vitamin retention: Canned peas. *J. Am. Dietet. A.,* 1945, *21:* 700–702.

FILER, L. J. JR., MATTIL, K. F., AND LONGENECKER, H. E. Changes occurring in fat autoxidation. *Oil & Soap,* 1945, *22:* 196–201.

FILER, L. J. JR., MATTIL, K. F., AND LONGENECKER, H. E. Antioxidant losses during the induction period of fat oxidation. *Oil & Soap,* 1944, *21:* 289–292.

GLEIM, E., ALBURY, M., VISNYEI, K., MCCARTNEY, J. R., AND FENTON, F. Retention of carotene, thiamin, riboflavin, and niacin during large-scale food service of dehydrated carrots. *Food Research,* 1946, *11:* 61–68.

GLEIM, E., ALBURY, M., VISNYEI, K., McCARTNEY, J. R., AND FENTON, F. Effect of quantity preparation procedures on vitamin retention; Canned tomatoes. *J. Am. Dietet. A.*, 1946, 22: 29–31.

GOULD, B. S., AND SCHWACHMAN, H. A new method for the bioassay of antiscorbutic substances: Assays of dehydroascorbic acid, 2-ketogulonic acid, iron ascorbate, and the effectiveness of oral and parenteral administration of ascorbic acid. *J. Biol. Chem.*, 1943, 151: 439–453.

GOVIER, W. M., AND GREIG, M. E. Prevention of oral lesions in B_1 avitaminotic dogs. *Science*, 1943, 98: 216–217.

GUETZKOW, H., HENSCHEL, A., AND BROZEK, J. Relationship of "psychoneurotic" changes to carbohydrate utilization in men on experimentally varied intake of B-complex vitamins. *Federation Proc.*, 1946, 5: 38.

GUETZKOW, H., TAYLOR, H. L., BROZEK, J., AND KEYS, A. Relationship of speed of motor reaction to blood sugar level during acute starvation in man. *Federation Proc.*, 1945, 4: 28.

HEGSTED, D. M., AND STARE, F. J. Nutritional studies with the duck. I. Purified rations for the duck. *J. Nutrition*, 1945, 30: 37–44.

HENSCHEL, A. The deterioration of brief endurance work capacity during semistarvation. *Federation Proc.*, 1946, 5: 45.

HENSCHEL, A., AND STURGEON, A. M. The effect of semi-starvation on the emptying of the human stomach. *Federation Proc.*, 1946, 5: 45.

HENSCHEL, A., TAYLOR, H. L., MICKELSEN, O., AND KEYS, A. Blood lactate and pyruvate relations in man. *Federation Proc.*, 1945, 4: 33.

HUFF, J. W., AND PERLZWEIG, W. A. A product of oxidative metabolism of pyridoxine, 2-methyl-3-hydroxy-4-carboxy-5-hydroxy-methylpyridine (4-pyridoxic acid). I. Isolation from urine, structure, and synthesis. *J. Biol. Chem.*, 1944, 155: 345–355.

HUFF, J. W., PERLZWEIG, W. A., AND TILDEN, M. W. A simple method for the determination of N'-methylnicotinamide in urine. *Federation Proc.*, 1945, 4: 92–93.

JOHNSON, R. E. A field nutritional laboratory. *War Medicine*, 1945, 7: 222–226.

JOHNSON, R. E., CONTRERAS, L. A., CONSOLAZIO, F. C., AND ROBINSON, P. F. A comparison of intravenous and oral vitamin tolerance tests. *Am. J. Physiol.*, 1945, 144: 58–68.

JOHNSON, R. E., DARLING, R. C., SARGENT, F., AND ROBINSON, P. Effects of variations in dietary vitamin C on the physical well being of manual workers. *J. Nutrition*, 1945, 29: 155–165.

JOHNSON, R. E., HENDERSON, C., ROBINSON, P. F., AND CONSOLAZIO, F. C. Comparative merits of fasting specimens, random specimens and oral loading tests in field nutritional surveys. *J. Nutrition*, 1945, 30: 89–98.

JOHNSON, R. E., SARGENT, F., ROBINSON, P. F., AND CONSOLAZIO, F. C. Estimation of riboflavin, thiamine, and N'-methylnicotinamide. Rapid field methods. *Indust. & Engin. Chem.*, 1945, 17: 384–386.

JOHNSON, R. E., SARGENT, F., ROBINSON, P. F., AND CONSOLAZIO, F. C. Assessment of nutritional and metabolic condition in the field. General and clinical aspects. *War Medicine*, 1945, 7: 227–233.

KEYS, A. Experimental human starvation — general and metabolic results of a loss of one-fourth the body weight in six months. *Federation Proc.*, 1946, 5: 55.

KEYS, A., AND HENSCHEL, A. Vitamin supplementation of U. S. Army rations in relation to fatigue and ability to do muscular work. *J. Nutrition*, 1942, 23: 259–269.

KEYS, A., HENSCHEL, A., MICKELSEN, O., AND BROZEK, J. M. The performance of normal young men on controlled thiamine intakes. *J. Nutrition*, 1943, 26: 399–415.

KEYS, A., HENSCHEL, A., MICKELSEN, O., BROZEK, J. M., AND CRAWFORD, J. H. Physiological and biochemical functions in normal young men on a diet restricted in riboflavin. *J. Nutrition*, 1944, 27: 165–178.

KEYS, A., HENSCHEL, A., TAYLOR, H. L., MICKELSEN, O., AND BROZEK, J. Experimental studies on man with a restricted intake of the B vitamins. I. The borderline of deficiency. II. The results of acute deprivation of vitamins of the B complex. *Am. J. Physiol.*, 1945, 144: 5–42.

KEYS, A., HENSCHEL, A., TAYLOR, H. L., MICKELSEN, O., AND BROZEK, J. Absence of rapid deterioration in men doing hard physical work on a restricted intake of vitamins of the B complex. *J. Nutrition*, 1944, 27: 485–496.

KEYS, A., HENSCHEL, A., TAYLOR, H. L., MICKELSEN, O., AND BROZEK, J. Hard work on restricted B vitamins. *Federation Proc.*, 1944, 3: 25.

KOCH, F. G., STREIGHTOFF, F., MUNSELL, H. E., BEN-DOR, BEN-AMI, ORR, M. L., CAILLEAU, R., LEONARD, M. H., EZEKIEL, S. R., AND KORNBLUM, R. The effect of large-scale methods of preparation on the vitamin content of food. I. The ascorbic acid, niacin, thiamin, and riboflavin content of potatoes. Headquarters, Military District of Washington, Office of the Pentagon Post Restaurant Officer, Nutrition Laboratory. "Publication No 1, 1 Aug. 1945."

LOWRY, O. H., AND BESSEY, O. A. The effects of light, trauma, riboflavin, and ariboflavinosis on the production of corneal vascularity and on healing of corneal lesions. *J. Nutrition*, 1945, 30: 285–292.

MATTIL, K. F., AND FILER, L. J. JR. Determination of gallic acid added to fats and oils. *Indust. & Engin. Chem.*, 1944, 16: 427–429.

MATTIL, K. F., FILER, L. J., JR., AND LONGENECKER, H. E. A study of the antioxidant effectiveness of several compounds on vegetable fats and oils. *Oil & Soap*, 1944, 21: 160–161.

MICKELSON, O. The urinary excretion of thiamine, pyramin (the pyrimidine-like component of thiamine) and riboflavin by man in semi-starvation. *Federation Proc.*, 1946, 5: 73.

MICKELSEN, O., DOEDEN, D., AND KEYS, A. The urinary excretion of riboflavin by man as related to diet and physical activity. *Federation Proc.*, 1945, 4: 98–99.

MICKELSEN, O., AND ERICKSON, L. L. The F_2 excretion of normal men on different levels of niacin intake. *Proc. Soc., Exper. Biol. & Med.*, 1945, 58: 33–36.

MICKELSEN, O., AND KEYS, A. The composition of sweat, with special reference to the vitamins. *J. Biol. Chem.*, 1943, 149: 479–490.

MORGAN, A. F., CARL, B. C., HUNNER, M. C., KIDDER, L. E., HUMMEL, M., AND PEAT, J. M. Vitamin losses in commercially produced dehydrated vegetables, cabbage, potatoes, carrots, and onions. *Fruit Products J. & Am. Food Manufacturer*, 1944, 23: 207–211.

MURLIN, J. R., EDWARDS, L. E., AND HAWLEY, E. E. Biological values and true digestibilities of some food proteins determined on human subjects. *J. Biol. Chem.*, 1944, 156: 785–786.

PERLZWEIG, W. A., AND HUFF, J. W. The fate of n'-methylnicotinamide in man. *J. Biol. Chem.,* 1945, *161:* 417–418.

PERLZWEIG, W. A., HUFF, J. W., AND GUE, I. The excretion of thiamin, riboflavin, and nicotinic acid by fasting men. *Federation Proc.,* 1944, *3:* 62.

PERLZWEIG, W. A., KAMIN, H., GUE, I., AND BLALOCK, J. V. A modification of the thiochrome method for the rapid determination of thiamine in urine. *Arch. Biochem.,* 1945, *6:* 97–103.

PERLZWEIG, W. A., RUFFIN, J. M., AND CAYER, D. Excretion of orally administered B-vitamins in health and disease. *Federation Proc.,* 1945, *4:* 161.

SARGENT, F., ROBINSON, P., AND JOHNSON, R. E. F_1 and F_2 of Najjar and Holt in the urine of normal young men. *J. Clin. Investigation,* 1944, *23:* 714–719.

SIMONSON, E. Electrocardiographic changes in semi-starvation. *Federation Proc.,* 1946, *5:* 95.

SIMONSON, E., AND BROZEK, J. Maximum speed of movement as a test of muscle function in different nutritional states. *Federation Proc.,* 1946, *5:* 96.

SIMONSON, E., HENSCHEL, A., TAYLOR, H. L., AND KEYS, A. Electrocardiographic changes in different nutritional states. *Federation Proc.,* 1945, *4:* 65–66.

SOBOTKA, H., BLOCH, E., CAHNMANN, H., FELDBAU, E., AND ROSEN, E. Studies on ionone. II. Optical resolution of dl-alpha-ionone. *J. Am. Chem. Soc.,* 1943, *65:* 2061–2062.

SOBOTKA, H., BLOCH, E., AND GLICK, D. Studies on ionone. I. Cleavage of ethyl ionylidene acetate. *J. Am. Chem. Soc.,* 1943, *65:* 1961–1963.

SOBOTKA, H., DARBY, H. H., GLICK, D., AND BLOCH, E. Studies on ionone. III. Structure of ethyl ionylidene-acetates. *J. Am. Chem. Soc.,* 1945, *67:* 403–406.

STARE, F. J., AND THORN, G. W. Some medical aspects of protein foods. *Am. J. Pub. Health,* 1943, *33:* 1444–1450.

TAYLOR, H. L. The effect of six months of semi-starvation on the maximal oxygen intake. *Federation Proc.,* 1946, *5:* 104.

TAYLOR, H. L., BROZEK, J., HENSCHEL, A., MICKELSEN, O., AND KEYS, A. The effect of successive fasts on the ability of men to withstand fasting during hard work. *Am. J. Physiol.,* 1945, *143:* 148–155.

Acclimatization

ADOLPH, E. F. Dehydration produces intolerance to heat. *Federation Proc.,* 1945, *4:* 1.

ADOLPH, E. F. The initiation of sweating in response to heat. *Am. J. Physiol.,* 1946, *145:* 710–715.

ADOLPH, E. F., BROWN, A. H., AND RAHN, H. Dehydration exhaustion. *Federation Proc.,* 1944, *3:* 1.

ADOLPH, E. F., AND ROTHSTEIN, A. Voluntary dehydration. *Federation Proc.,* 1944, *3:* 1.

FOURT, L. Significance of water vapor permeability of textile fabrics. Symposium on the functional properties of clothing fabrics issued by Textile Research Inst., New York, 1943, pp. 8–12.

GOSSELIN, R. E. Heat gains by man in the desert. Federation Proc., 1945, *4:* 25.

HENSCHEL, A., TAYLOR, H. L., BROZEK, J., MICKELSEN, O., AND KEYS, A. Vita-

min C and ability to work in hot environments. *Am. J. Trop. Med.,* 1944, *24:* 259–265.

HENSCHEL, A., TAYLOR, H. L., AND KEYS, A. The gastric emptying time of man at high and normal environmental temperatures. *Am. J. Physiol.,* 1944, *141:* 205–208.

HENSCHEL, A., TAYLOR, H. L., AND KEYS, A. The persistence of heat acclimatization in man. *Am. J. Physiol.,* 1943, *140:* 321–325.

HENSCHEL, A., TAYLOR, H. L., MICKELSEN, O., AND KEYS, A. The effect of high vitamin C and B vitamin intakes on the ability of man to work in hot environments. *Federation Proc.,* 1944, *3:* 18.

JOHNSON, B. C. Microbiological differentiation of pyridoxine and pseudopyridoxine. *Proc. Soc. Exper. Biol. & Med.,* 1944, *55:* 199–201.

JOHNSON, B. C., HAMILTON, T. S., AND MITCHELL, H. H. The effect of choline intake and environmental temperature on the excretion of choline from the human body. *J. Biol. Chem.,* 1945, *159:* 5–8.

JOHNSON, B. C., HAMILTON, T. S., AND MITCHELL, H. H. The excretion of nicotinic acid, nicotinamide, nicotinuric acid, and (N'-methylnicotinamide) by normal individuals. *J. Biol. Chem.,* 1945, *159:* 231–236.

JOHNSON, B. C., HAMILTON, T. S., AND MITCHELL, H. H. The excretion of "folic acid" through the skin and in the urine of normal individuals. *J. Biol. Chem.,* 1945, *159:* 425–429.

JOHNSON, B. C., HAMILTON, T. S., AND MITCHELL, H. H. The excretion of pyridoxine "pseudopyridoxine," and 4-pyridoxic acid in the urine and sweat of normal individuals. *J. Biol. Chem.,* 1945, *158:* 619–623.

JOHNSON, B. C., MITCHELL, H. H., AND HAMILTON, T. S. The occurrence of inositol and of p-aminobenzoic acid in sweat. *J. Biol. Chem.,* 1945, *161:* 357–360.

JOHNSON, R. E. Nutritional standards for men in tropical climates. *Gastroenterol.,* 1943, *1:* 832–840.

JOHNSON, R. E., PITTS, G. C., AND CONSOLAZIO, F. C. Factors influencing chloride concentration in human sweat. *Am. J. Physiol.,* 1944, *141:* 575–589.

McCLURE, F. J. Nondental physiological effects of trace quantities of fluorine. *J. Am. Coll. Dentists,* 1945, *12:* 58–60.

McCLURE, F. J., MITCHELL, H. H., HAMILTON, T. S., AND KINSER, C. A. Balances of fluorine ingested from various sources in food and water by five young men. Excretion of fluorine through the skin. *J. Indust. Hyg. & Toxicol.,* 1945, *27:* 159–170.

MOLNAR, G. W., TOWBIN, E. J., AND BROWN, A. H. Environmental heat gain of man in hot humid climate. *Federation Proc.,* 1945, *4:* 52.

MOREIRA, M., JOHNSON, R. E., FORBES, A. P., AND CONSOLAZIO, F. Adrenal cortex and work in the heat. *Am. J. Physiol.,* 1945, *143:* 169–176.

PITTS, G. C., CONSOLAZIO, F. C., AND JOHNSON, R. E. Dietary protein and physical fitness in temperate and hot environments. *J. Nutrition,* 1944, *27:* 497–508.

PITTS, G. C., JOHNSON, R. E., AND CONSOLAZIO, F. C. Work in the heat as affected by intake of water, salt and glucose. *Am. J. Physiol.,* 1944, *142:* 253–259.

ROBINSON, S., TURRELL, E. S., BELDING, H. S., AND HORVATH, S. M. Rapid acclimatization to work in hot climates. *Am. J. Physiol.,* 1943, *140:* 168–176.

ROBINSON, S., TURRELL, E. S., AND GERKING, S. D. Physiologically equivalent conditions of air temperature and humidity. *Am. J. Physiol.,* 1945, *143:* 21–32.

SARGENT, F., ROBINSON, P., AND JOHNSON, R. E. Water-soluble vitamins in sweat. *J. Biol. Chem.*, 1944, *153:* 285–294.

SHIELDS, J. B., JOHNSON, B. C., HAMILTON, T. S., AND MITCHELL, H. H. The excretion of ascorbic acid and dehydroascorbic acid in sweat and urine under different environmental conditions. *J. Biol. Chem.*, 1945, *161:* 351–356.

SPECTOR, H., AND HAMILTON, T. S. The microdetermination of iodine in biological materials with special reference to the combustion of samples in the Parr oxygen bomb. *J. Biol. Chem.*, 1945, *161:* 127–135.

SPECTOR, H. HAMILTON, T. S., AND MITCHELL, H. H. The effect of pantothenic acid dosage and environmental temperature and humidity upon the dermal and renal excretion of pantothenic acid. *J. Biol. Chem.*, 1945, *161:* 145–152.

SPECTOR, H., MITCHELL, H. H., AND HAMILTON, T. S. The effect of environmental temperature and potassium iodide supplementation on the excretion of iodine by normal human subjects. *J. Biol. Chem.*, 1945, *161:* 137–143.

TAYLOR, H. L. Some common misconceptions concerning the physiology of high temperatures. *Bull. Minnesota M. Found.*, 1944, *4:* 90–93.

TAYLOR, H. L., HENSCHEL, A., AND KEYS, A. Cardiovascular adjustments of man in rest and work during exposure to dry heat. *Am. J. Physiol.*, 1943, *139:* 583–591.

TAYLOR, H. L., HENSCHEL, A., MICKELSEN, O., AND KEYS, A. The effect of the sodium chloride intake on the work performance of man during exposure to dry heat and experimental heat exhaustion. *Am. J. Physiol.*, 1943, *140:* 439–451.

WILLS, J. H. Water drinking by dehydrated men in desert environment. *Federation Proc.*, 1945, *4:* 77–78.

WILLS, J. H., AND KELLY, J. J. Urinary and salivary flows under desert conditions. *Federation Proc.*, 1944, *3:* 51–52.

Miscellaneous Physiological Studies

BRANNON, E. S., MERRILL, A. J., WARREN, J. V., AND STEAD, E. A. JR. The cardiac output in patients with chronic anemia as measured by the technique of right atrial catheterization. *J. Clin. Investigation*, 1945, *24:* 332–336.

BROWN, A. H., AND GOSSELIN, R. E. Water losses of men on life rafts. *Federation Proc.*, 1944, *3:* 5–6.

COURNAND, A. Measurement of the cardiac output in man using the right heart catheterization. Description of technique, discussion of validity and of place in the study of the circulation. *Federation Proc.*, 1945, *4:* 207–212.

COURNAND, A., LAUSON, H. D., BLOOMFIELD, R. A., BREED, E. S., AND BALDWIN, E. DEF. Recording of right heart pressures in man. *Proc. Soc., Exper. Biol. & Med.*, 1944, *55:* 34–36.

COURNAND, A., RILEY, R. L., BREED, E. S., BALDWIN, E. DEF., AND RICHARDS, D. W. JR. Measurement of cardiac output in man using the technique of catheterization of the right auricle or ventricle. *J. Clin. Investigation*, 1945, *24:* 106–116.

CRESCITELLI, F., AND TAYLOR, C. The lactate response to exercise and its relationship to physical fitness. *Am. J. Physiol.*, 1944, *141:* 630–640.

GAMBLE, J. L. The water requirements of castaways. *Proc. Am. Philosophical Soc.,* 1944, *88:* 151–158.

GAMBLE, J. L., AND BUTLER, A. M. Measurement of renal water requirement. *Tr. A. Am. Physicians,* 1944, *58:* 157–161.

RICHARDS, D. W. JR. Cardiac output by the catheterization technique in various clinical conditions. *Federation Proc.,* 1945, *4:* 215–220.

STEAD, E. A. JR., WARREN, J. V., MERRILL, A. J., AND BRANNON, E. S. The cardiac output in male subjects as measured by the technique of right atrial catheterization. Normal values with observations on the effect of anxiety and tilting. *J. Clin. Investigation,* 1945, *24:* 326–331.

TAYLOR, C. A maximal pack test of exercise tolerance. *Research Quarterly,* 1944, Dec.: 291–302.

TAYLOR, C. Some properties of maximal and submaximal exercise with reference to physiological variation and the measurement of exercise tolerance. *Am. J. Physiol.,* 1944, *142:* 200–212.

TAYLOR, C., AND BROWN, G. E. JR. Some observations on the validity of the Schneider test. *J. Aviation Med.,* 1944, *15:* 214–230.

TYLER, D. B., MARX, W., AND GOODMAN, J. Effect of prolonged wakefulness on the urinary excretion of 17-ketosteroids. *Proc. Soc. Exper. Biol. & Med.,* 1946, *62:* 38–40.

WARREN, J. V., STEAD, E. A. JR., AND BRANNON, E. S. The cardiac output in man: A study of some of the errors in the method of right heart catheterization. *Am. J. Physiol.,* 1946, *145:* 458–464.

CHEMISTRY

Treatment of Gas Casualties

FRIEDENWALD, J. S., HUGHES, W. F. JR., AND HERMANN, H. Acid-base tolerance of the cornea. *Arch. Ophth.,* 1944, *31:* 279–283.

SULZBERGER, M. B., AND KATZ, J. H. The absence of skin irritants in the contents of vesicles. *U. S. Nav. M. Bull.,* 1943, *41:* 1258–1262.

WOODS, A. C., AND HUGHES, W. F. JR. Chemical burns of eyes. *Military Surgical Manuals, Ophthalmology and Otolaryngology,* Philadelphia, W. B. Saunders Co., 1942, pp. 61–66.

Insect Control

BUSHLAND, R. C., MCALISTER, L. C. JR., EDDY, G. W., AND JONES, H. A. DDT for the control of human lice. *J. Economic Entomology,* 1944, *37:* 126–127.

BUSHLAND, R. C., MCALISTER, L. C. JR., JONES, H. A., AND CULPEPPER, G. H. DDT powder for the control of lice attacking man. *J. Economic Entomol.,* 1945, *38:* 210–217.

BUSHLAND, R. C., MCALISTER, L. C. JR., EDDY, G. W., JONES, H. A., AND KNIPLING, E. F. Development of a powder treatment for the control of lice attacking man. *J. Parasitol.,* 1944, *30:* 377–387.

BUSHLAND, R. C., EDDY, G. W., AND KNIPLING, E. F. Tests with synergists for pyrethrum against the body louse. *J. Economic Entomol.,* 1944, *37:* 556.

BUSHLAND, R. C., SCHECHTER, M. S., JONES, H. A., AND KNIPLING, E. F. Antioxidants for pyrethrum powders. *Soap & Sanitary Chemicals,* 1945, November.

CARPENTER, C. C., HEINLEIN, J. A., SULZBERGER, M. B., AND BAER, R. L. Scabies and pediculosis treated with benzyl benzoate, DDT, benzocaine emulsion. *J. Invest. Dermat.,* 1946, *7:* 93–98.

CRISTOL, S. J. A kinetic study of the dehydrochlorination of substituted 2,2-diphenylchloroethanes related to DDT. *J. Am. Chem. Soc.,* 1945, *67:* 1494–1498.

CRISTOL, S. J., AND HALLER, H. L. The chemistry of DDT—a review. *Chem. & Engin. News,* 1945, *23:* 2070–2075.

CRISTOL, S. J., AND HALLER, H. L. Bromine analogs of DDT. *J. Am. Chem. Soc.,* 1946, *68:* 140–141.

CRISTOL, S. J., AND HALLER, H. L. Dehydrochlorination of 1-trichloro-2-o-chlorophenyl-2-chlorophenylethane (o,p′-DDT isomer). *J. Am. Chem. Soc.,* 1945, *67:* 2222–2223.

CRISTOL, S. J., HAYES, R. A., AND HALLER, H. L. Determination of 1-trichloro-2,-2-bis (p-chlorophenyl) ethane in technical DDT. *Indust. & Engin. Chem.,* 1945, *17:* 470–472.

CULPEPPER, G. H. The rearing and maintenance of a laboratory colony of the body louse. *Am. J. Trop. Med.,* 1944, *24:* 327–329.

DEONIER, C. C. AND BURRELL, R. W. Airplane application of DDT larvicides. *J. Economic Entomol.,* 1945, *38:* 425–427.

DEONIER, C. C., BURRELL, R. W., MAPLE, J. D., AND COCHRAN, J. H. DDT as an anopheline larvicide: Preliminary field studies. *J. Economic Entomol.,* 1945, *38:* 244–249.

DEONIER, C. C., MAPLE, J. D., JONES, H. A., HINCHEY, E., AND EIDE, P. M. DDT as an anopheline larvicide-laboratory test. *J. Economic Entomol.,* 1945, *38:* 241–243.

DRAIZE, J. H., NELSON, A. A., AND CALVERY, H. O. The percutaneous absorption of DDT (2,2-bis (p-chlorophenyl) 1,1,1-trichloroethane) in laboratory animals. *J. Pharmacol. & Exper. Therap.,* 1944, *82:* 159–166.

DRAIZE, J. H., WOODWARD, G., FITZHUGH, O. G., NELSON, A. A., SMITH, R. B. JR., AND CALVERY, H. O. Summary of toxicological studies of the insecticide DDT, 2,2-bis (p-chlorophenyl) 1,1,1-trichloroethane. *Chem. & Engin. News,* 1944, *22:* 1503.

EDDY, G. W. A combination treatment for lice and scabies. *J. Invest. Dermat.,* 1946, *7:* 85–91.

EDDY, G. W. A treatment for head lice, crab lice, and scabies. *War Medicine,* 1944, *6:* 319–322.

EIDE, P. M., DEONIER, C. C., AND BURRELL, R. W. DDT as a culicine larvicide. *J. Economic Entomol.,* 1946, *38:* 537–541.

EIDE, P. M., DEONIER, C. C., AND BURRELL, R. W. The toxicity of DDT to certain forms of aquatic life. *J. Economic Entomol., 38:* 492–493.

GAHAN, J. B., AND KNIPLING, E. F. Efficacy of DDT as a roach poison. *J. Economic Entomol.,* 1944, *37:* 138–139.

GAHAN, J. B., AND LINDQUIST, A. W. DDT residual sprays applied in buildings to control Anopheles quadrimaculatus. *J. Economic Entomol.,* 1945, *38:* 223–230.

GAHAN, J. B., TRAVIS, B. V., MORTON, F. A., AND LINDQUIST, A. W. DDT as a

residual-type treatment to control Anopheles quadrimaculatus: Practical tests. *J. Economic Entomol.*, 1945, *38*: 231–235.

GAHAN, J. B., TRAVIS, B. V., AND LINDQUIST, A. W. DDT as a residual-type spray to control disease-carrying mosquitoes: Laboratory tests. *J. Economic Entomol.*, 1945, *38*: 236–240.

HALL, S. A., SCHECHTER, M. S., AND FLECK, E. E. Chemical determination of DDT. U. S. Department of Agriculture, Bureau of Entomology & Plant Quarantine, Publication ET–211, 1944, November.

JONES, H. A., McALISTER, L. C. JR., BUSHLAND, R. C., AND KNIPLING, E. F. Experimental impregnation of underwear with pyrethrum extract for control of body lice. *War Medicine*, 1944, *6*: 323–326.

JONES, H. A., McALISTER, L. C. JR., BUSHLAND, R. C., AND KNIPLING, E. F. DDT impregnation of underwear for control of body lice. *J. Economic Entomol.*, 1945, *38*: 217–223.

JONES, H. A., DEONIER, C. C., BURRELL, R. W., AND KNIPLING, E. F. Larvicidal aerosols containing DDT. *J. Economic Entomol.*, 1945, *38*: 432–433.

JONES, H. A., FLUNO, H. T., AND McCOLLOUGH, G. T. Solvents for DDT. Soap & Sanitary Chemicals, 1945, November.

JONES, H. A., FLUNO, H. T., AND HENDRICK, A. B. DDT insecticidal preparations. *J. Economic Entomol.*, 1945, *38*: 207–210.

KNIPLING, E. F. DDT insecticides developed for use by the armed forces. *J. Economic Entomol.*, 1945, *38*: 205–207.

KNIPLING, E. F., AND DOVE, W. E. Recent investigations of insecticides and repellents for the armed forces. *J. Economic Entomol.*, 1944, *37*: 477–480.

LAUG, E. P., AND FITZHUGH, O. G. 2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane (DDT) in the tissues of the rat following oral ingestion for periods of six months to two years. *J. Pharmacol. & Exper. Therap.*, 1946, *87*: 18–23.

LINDQUIST, A. W., JONES, H. A., AND MADDEN, A. H. DDT residual-type sprays as affected by light. *J. Economic Entomol.*, 1946, *39*: 55–59.

LINDQUIST, A. W., KNIPLING, E. F., JONES, H. A., AND MADDEN, A. H. Mortality of bedbugs on rabbits given oral dosages of DDT and pyrethrum. *J. Economic Entomol.*, 1944, *37*: 128.

LINDQUIST, A. W., MADDEN, A. H., HUSMAN, C. N., AND TRAVIS, B. V. DDT dispersed from airplanes for control of adult mosquitoes. *J. Economic Entomol.*, 1946, *38*: 541–544.

LINDQUIST, A. W., MADDEN, A. H., AND KNIPLING, E. F. DDT as a treatment for fleas on dogs. *J. Economic Entomol.*, 1944, *37*: 138.

LINDQUIST, A. W., MADDEN, A. H., AND WATTS, C. N. The use of repellents against fleas. *J. Economic Entomol.*, 1944, *37*: 485–486.

LINDQUIST, A. W., MADDEN, A. H., WILSON, H. G., AND JONES, H. A. The effectiveness of DDT as a residual spray against houseflies. *J. Economic Entomol.*, 1944, *37*: 132–134.

LINDQUIST, A. W., MADDEN, A. H., WILSON, H. G., AND KNIPLING, E. F. DDT as a residual-type treatment for control of houseflies. *J. Economic Entomol.*, 1945, *38*: 257–261.

LINDQUIST, A. W., AND McDUFFIE, W. C. DDT-oil sprays applied from an airplane to control Anopheles and Mansonia mosquitoes. *J. Economic Entomol.*, 1946, *38*: 545–548.

LINDQUIST, A. W., AND McDUFFIE, W. C. DDT residual spray tests in Panama. *J. Economic Entomol.,* 1946, *38:* 608.

LINDQUIST, A. W., SCHROEDER, H. O., AND KNIPLING, E. F. Concentrated insecticides: Preliminary studies of the use of concentrated sprays against houseflies and mosquitoes. *Soap & Sanitary Chemicals,* 1945, July.

LINDQUIST, A. W., TRAVIS, B. V., MADDEN, A. H., SCHROEDER, H. O., AND JONES, H. A. DDT and pyrethrum aerosols to control mosquitoes and houseflies under semi-practical conditions. *J. Economic Entomol.,* 1945, *38:* 255–257.

LINDQUIST, A. W., WILSON, H. G., SCHROEDER, H. O., AND MADDEN, A. H. Effect of temperatures on knockdown and kill of houseflies exposed to DDT. *J. Economic Entomol.,* 1945, *38:* 261–264.

MADDEN, A. H., LINDQUIST, A. W., AND KNIPLING, E. F. DDT and other insecticides as residual-type treatments to kill bedbugs. *J. Economic Entomol.,* 1945, *38:* 265–271. DDT as a residual spray for the control of bedbugs. *J. Economic Entomol.,* 1944, *37:* 127–128.

MADDEN, A. H., LINDQUIST, A. W., AND KNIPLING, E. F. DDT treatment of airplanes to prevent introduction of noxious insects. *J. Economic Entomol.,* 1945, *38:* 252–254.

MADDEN, A. H., LINDQUIST, A. W., AND KNIPLING, E. F. Tests of repellents against chiggers. *J. Economic Entomol.,* 1944, *37:* 283–286.

NELSON, A. A., DRAIZE, J. H., WOODARD, G., FITZHUGH, O. G., SMITH, R. B., JR., AND CALVERY, ·H. O. Histopathological changes following administration of DDT to several species of animals. *Pub. Health Rep.,* 1944, *59:* 1009–1020.

OFNER, ʹR. R., AND CALVERY, H. O. Determination of DDT (2,2-bis (p-chlorophenyl) 1,1,1-trichloroethane) and its metabolite in biological materials by use of the Schechter-Haller method. *J. Pharmacol. & Exper. Therap.,* 1945, *85:* 363–370.

RICHARDS, A. G. JR. The structure of living insect nerves and nerve sheaths as deduced from the optical properties. *J. New York Entomol. Soc.,* 1944, *52:* 285–310.

RICHARDS, A. G. JR., AND CUTKOMP, L. K. Correlation between the possession of a chitinous cuticle and sensitivity to DDT. *Biol. Bull.,* 1946, *90:* 97–108.

RICHARDS, A. G. JR., AND CUTKOMP, L. K. Neuropathology in insects. *J. New York Entomol. Soc.,* 1945, *53:* 313–355.

RICHARDS, A. G. JR., AND CUTKOMP, L. K. The cholinesterase of insect nerves. *J. Cell. & Comp. Physiol.,* 1945, *26:* 57–61.

RICHARDS, A. G. JR., AND WEYGANDT, J. L. The selective penetration of fat solvents into the nervous system of mosquito larvae. *J. New York Entomol. Soc.,* 1945, *53:* 153–165.

SCHECHTER, M. S., SOLOWAY, S. B., HAYES, R. A., AND HALLER, H. L. Colorimetric determination of DDT. Color test for related compounds. *Indust. & Engin. Chem.,* 1945, *17:* 704–709.

SCHROEDER, H. O., MADDEN, A. H., WILSON, H. G., AND LINDQUIST, A. W. Residual action of DDT aerosols against houseflies. *J. Economic Entomol.,* 1945, *38:* 277–278.

WISECUP, C. B., BURRELL, R. W., AND DEONIER, C. C. DDT sprays mechanically dispersed for control of anopheline mosquito larvae. *J. Economic Entomol.,* 1945, *38:* 434–436.

WISECUP, C. B., AND DEONIER, C. C. DDT for the control of Psorophora mosquitoes. *J. Economic Entomol.*, 1945, *38:* 250–252.

WOODARD, G., NELSON, A. A., AND CALVERY, H. O. Acute and subacute toxicity of DDT (2,2,-bis(p-chlorophenyl)-1,1,1-trichloroethane) to laboratory animals. *J. Pharmacol. & Exper. Therap.*, 1944, *82:* 152–158.

WOODARD, G., OFNER, R. R. AND MONTGOMERY, C. M. Accumulation of DDT in the body fat and its appearance in the milk of dogs. *Science,* 1945, *102:* 177–178.

Rodent Control and Rat Bite Fever

ALTEMEIER, W. A., SNYDER, H., AND HOWE, G. Penicillin therapy in rat bite fever. *J.A.M.A.,* 1945, *127:* 270–273.

DIEKE, S. H., AND RICHTER, C. P. Acute toxicity of thiourea to rats in relation to age, diet, strain and species variation. *J. Pharmacol. & Exper. Therap.,* 1945, *83:* 195–202.

DIEKE, S. H., AND RICHTER, C. P. Age and species variation in the acute toxicity of alpha-naphthyl thiourea. *Proc. Soc. Exper. Biol. & Med.* 1946, *62:* 22–25.

EMLEN, J. T. JR. Device for holding live wild rats. *J. Wildlife Management,* 1944, *8:* 264–265.

KALMBACH, E. R. "Ten-eighty," a war-produced rodenticide. *Science,* 1945, *102:* 232–233.

RICHTER, C. P. The development and use of alpha-naphthyl thiourea (ANTU) as a rat poison. *J.A.M.A.,* 1945, *129:* 927–931.

RICHTER, C. P. Incidence of rat bites and rat bite fever in Baltimore. *J.A.M.A.,* 1945, *128:* 324–326.

RICHTER, C. P., AND EMLEN, J. T. JR. A modified rabbit box trap for use in catching live wild rats for laboratory and field studies. *Pub. Health Rep.,* 1945, *60:* 1303–1308.

WARD, J. C. Rodenticides — present and future. *Soap & Sanitary Chemicals,* 1945, September.

WARD, J. C. Rat control — the use of rodenticides. *Pests & Their Control,* 1945, October.

ADRENAL CORTICAL HORMONES

CLINTON, M. JR., AND THORN, G. W. Effect of desoxycorticosterone acetate administration on plasma volume and electrolyte balance of normal human subjects. *Bull. Johns Hopkins Hosp.,* 1943, *72:* 255–264.

CLINTON, M. JR., THORN, G. W., EISENBERG, H., AND STEIN, K. E. Effect of synthetic desoxycorticosterone acetate therapy on plasma volume and electrolyte balance in normal dogs. *Endocrinology,* 1942, *31:* 578–581.

EVANS, H. M., SIMPSON, M. E., AND LI, C. H. Inhibiting effect of adrenocorticotropic hormone on the growth of male rats. *Endocrinology,* 1943, *33:* 237–238.

FIESER, L. F., FIELDS, M., AND LIEBERMAN, S. Concerning the characterization of possible cortical hormone metabolites in urine. *J. Biol. Chem.,* 1944, *156:* 191–201.

GALLAGHER, T. F. Partial synthesis of compounds related to adrenal cortical hormones. I. The structure of delta 11-lithocholenic acid. *J. Biol. Chem.*, 1946, *162:* 491–493.

GALLAGHER, T. F. Partial synthesis of compounds related to adrenal cortical hormones. VI. The structure of the "3(alpha), 11-dihydroxy-12-ketocholanic acid" of Marker and Lawson and of the products obtained by "Wolff-Kishner reduction." *J. Biol. Chem.*, 1946, *162:* 539–548.

GALLAGHER, T. F., AND HOLLANDER, V. P. Partial synthesis of compounds related to adrenal cortical hormones. V. Preparation of 3(alpha), 11(alpha)-dihydroxy-12-ketocholanic acid and the products obtained by Wolff-Kishner reduction. *J. Biol. Chem.*, 1946, *162:* 533–538.

GALLAGHER, T. F., AND LONG, W. P. Partial synthesis of compounds related to adrenal cortical hormones. IV. An improved method for the preparation of 3(alpha), 11(alpha)-dihydroxycholanic acid. *J. Biol. Chem.*, 1946, *162:* 521–532.

GALLAGHER, T. F., AND LONG, W. P. Partial synthesis of compounds related to adrenal cortical hormones. II. Some reactions of an epoxide of delta 11-lithocholenic acid. *J. Biol. Chem.*, 1946, *162:* 495–509.

HOLLANDER, V. P., AND GALLAGHER, T. F. Partial synthesis of compounds related to adrenal cortical hormones. VII. Degradation of the side chain of cholanic acid. *J. Biol. Chem.*, 1946, *162:* 549–553.

LI, C. H., SIMPSON, M. E., AND EVANS, H. M. Isolation of adrenocorticotropic hormone from sheep pituitaries. *Science,* 1942, *96:* 450.

LONG, W. P., AND GALLAGHER, T. F. Partial synthesis of compounds related to adrenal cortical hormones. III. Preparation of 3(alpha), 11 (alpha)-dihydroxycholanic acid. *J. Biol. Chem.*, 1946, *162:* 511–519.

OLSON, R. E., JACOBS, F. A., RICHERT, D., THAYER, S. A., KOPP, L. J., AND WADE, N. J. The comparative bioassay of several extracts of the adrenal cortex in tests employing four separate physiological responses. *Endocrinology,* 1944, *35:* 430–455.

OLSON, R. E., THAYER, S. A., AND KOPP, L. J. The glycogenic activity of certain crystalline steroids of the adrenal cortex when administered singly and with cortical extract to fasted, normal, and adrenalectomized rats. *Endocrinology,* 1944, *35:* 464–472.

SIMPSON, M. E., EVANS, H. M., AND LI, C. H. Bioassay of adrenocorticotropic hormone. *Endocrinology,* 1943, *33:* 261–268.

MALARIA

Clinical Studies

BRODIE, B. B., AND UDENFRIEND, S. The estimation of basic organic compounds and a technique for the appraisal of specificity. *J. Biol. Chem.*, 1945, *158:* 705–714.

BRODIE, B. B., AND UDENFRIEND, S. The estimation of atabrine in biological fluids and tissues. *J. Biol. Chem.*, 1943, *151:* 299–317.

BRODIE, B. B., AND UDENFRIEND, S. The estimation of quinine in human plasma with a note on the estimation of quinidine. *J. Pharmacol. & Exper. Therap.,* 1943, *78:* 154–158.

BRODIE, B. B., UDENFRIEND, S., AND COBURN, A. F. The determination of salicylic acid in plasma. *J. Pharmacol. & Exper. Therap.,* 1944, *80:* 114–117.

FELDMAN, H. A., AND MURPHY, F. D. The effect of alterations in blood volume on the anemia and hypoproteinemia of human malaria. *J. Clin. Investigation,* 1945, *24:* 780–792.

HEIDELBERGER, M., AND MAYER, M. M. Normal human stromata as antigens for complement fixation in the sera of patients with relapsing vivax malaria. *Science,* 1944, *100:* 359–360.

HIATT, E. P. Plasma concentrations following the oral administration of single doses of the principal alkaloids of cinchona bark. *J. Pharmacol. & Exper. Therap.,* 1944, *81:* 160–163.

HINDLE, J. A., ROSE, A. S., TREVETT, L. D., AND PROUT, C. The effect of penicillin on inoculation malaria. A negative report. *New England J. Med.,* 1945, *232:* 133–136.

PACKER, H. Recent advances in the chemotherapy of malaria. *J. Tennessee State M. A.,* 1946, *39:* 193–199.

SHANNON, J. A., AND EARLE, D. P. JR. Recent advances in the treatment of malaria. *Bull. New York Acad. Med.,* 1945, *21:* 467–481.

Mosquitoes' Feeding Habits

REEVES, W. C. Preliminary studies on the feeding habits of Pacific coast anophelines. *J. Nat. Malaria Soc.,* 1944, *3:* 261–266.

Parasitology

BALL, E. G., ANFINSEN, C. B., GEIMAN, Q. M., McKEE, R. W., AND ORMSBEE, R. A. In vitro growth and multiplication of the malaria parasite Plasmodium knowlesi. *Science,* 1945, *101:* 542–544.

CANTRELL, W., AND JORDAN, H. B. New mosquito hosts for Plasmodium gallinaceum. *J. Parasitol.,* 1945, *31:* 55–56.

CEITHAML, J., AND EVANS, E. A. JR. The biochemistry of the malaria parasite. IV. The in vitro effects of x-rays upon Plasmodium gallinaceum. *J. Infect. Dis.,* 1946, *78:* 190–197.

DEANE, H. W. Studies on malarial parasites: II. The staining of two primate parasites by the Feulgen technique. *J. Cell. & Comp. Physiol.,* 1945, *26:* 139–145.

FERREBEE, J. W., AND GEIMAN, Q. M. Studies on malarial parasites. III. A procedure for preparing concentrates of Plasmodium vivax. *J. Infect. Dis.,* 1946, *78:* 173–179.

FERREBEE, J. W., GIBSON, J. G., AND PEACOCK, W. C. Studies on malarial parasites. IV. Some observations regarding the age of the erythrocyte invaded by Plasmodium vivax. *J. Infect. Dis.,* 1946, *78:* 180–182.

HUFF, C. G., AND COULSTON, F. The development of Plasmodium gallinaceum from sporozoite to erythrocytic trophozoite. *J. Infect. Dis.,* 1944, *75:* 231–249.

HUFF, C. G., AND COULSTON, F. The relation of natural and acquired immunity of various avian hosts to the cryptozoites and metacryptozoites of Plasmodium gallinaceum and Plasmodium relictum. *J. Infect. Dis.*, 1946, *78:* 99–117.

SILVERMAN, M., CEITHAML, J., TALIAFERRO, L. G., AND EVANS, E. A. JR. The in vitro metabolism of Plasmodium gallinaceum. *J. Infect. Dis.*, 1944, *75:* 212–230.

SPECK, J. F., AND EVANS, E. A. JR. The biochemistry of the malaria parasite. II. Glycolysis in cell-free preparations of the malaria parasite. *J. Biol. Chem.*, 1945, *159:* 71–81.

SPECK, J. F., AND EVANS, E. A. JR. The biochemistry of the malaria parasite. III. The effects of quinine and atabrine on glycolysis. *J. Biol. Chem.*, 1945, *159:* 83–96.

WATSON, R. B. Observations on the transmissibility of strains of Plasmodium vivax from Pacific War Areas by Anopheles quadrimaculatus. *Am. J. Trop. Med.*, 1945, *25:* 315–321.

Pharmacology of Antimalarials

BRATTON, A. C. JR. A short-term chronic toxicity test employing mice. *J. Pharmacol. & Exper. Therap.*, 1945, *85:* 111–118.

BRATTON, A. C. JR. Continuous intravenous chemotherapy of Plasmodium lophurae infection in ducks. *J. Pharmacol. & Exper. Therap.*, 1945, *85:* 103–110.

BURTON, A. F., AND KELSEY, F. E. Studies on antimalarial drugs. The metabolism of quinine in pregnant animals. *J. Pharmacol. & Exper. Therap.*, 1943, *79:* 70–76.

BUTLER, T. C. The distribution of radiant energy in the fluorescent spectra of atabrine and some other derivatives of acridine. *J. Pharmacol. & Exper. Therap.*, 1944, *80:* 70–73.

CANTRELL, W., AND JORDAN, H. B. Changes in the infectiousness of gametocytes during the course of Plasmodium gallinaceum infections. *J. Infect. Dis.*, 1946, *78:* 153–159.

CHEN, G., AND GEILING, E. M. K. Observations bearing on the mechanism of the elimination of quinine and atabrine from the circulation and tissues. *J. Pharmacol. & Exper. Therap.*, 1944, *82:* 120–132.

CHEN, G., GEILING, E. M. K., AND MacHATTON, R. M. The effect of cysteine on the trypanocidal activity and toxicity of antimonials. *J. Infect. Dis.*, 1945, *76:* 152–154.

CHEN, G., GEILING, E. M. K., AND MacHATTON, R. M. Trypanocidal activity and toxicity of antimonials. *J. Infect. Dis.*, 1945, *76:* 144–151.

CHEN, G., GEILING, E. M. K., AND MacHATTON, R. M. Parasitemia and length of survival of mice infected with Trypanosoma equiperdum. *Am. J. Hyg.*, 1945, *41:* 221–227.

COATNEY, G. R., AND COOPER, W. C. The prophylactic effect of sulfadiazine and sulfaguanidine against mosquito-borne Plasmodium gallinaceum infection in the domestic fowl (preliminary report). *Pub. Health Rep.*, 1944, *59:* 1455–1458.

COATNEY, G. R., COOPER, W. C., AND MILES, V. I. Studies on Plasmodium gallinaceum Brumpt. I. The incidence and course of the infection in young chicks resulting from single mosquito bites. *Am. J. Hyg.*, 1945, *41:* 109–118.

COATNEY, G. R., COOPER, W. C., AND TREMBLEY, H. L. Studies on Plasmodium

gallinaceum Brumpt. II. The incidence and course of the infection in young chicks following the inoculation of infected salivary glands. *Am. J. Hyg.*, 1945, 41: 119–122.

COATNEY, G. R., COOPER, W. C., AND TREMBLEY, H. L. Studies on Plasmodium gallinaceum Brumpt. III. The incidence and course of the infection in young chicks following the subcutaneous inoculation of pooled sporozoites. *Am. J. Hyg.*, 1945, 42: 323–329.

COGGESHALL, L. T., PORTER, R. J., AND LAIRD, R. L. Prophylactic and curative effects of certain sulfonamide compounds on exoerythrocytic stages in Plasmodium gallinaceum malaria. *Proc. Soc. Exper. Biol. & Med.*, 1944, 57: 286–292.

COULSTON, F., CANTRELL, W., AND HUFF, C. G. The distribution and localization of sporozoites and pre-erythrocytic stages in infections with Plasmodium gallinaceum. *J. Infect. Dis.*, 1945, 76: 226–238.

DEARBORN, E. H., KELSEY, F. E., OLDHAM, F. K. AND GEILING, E. M. K. Studies on antimalarials. The accumulation and excretion of atabrine. *J. Pharmacol. & Exper. Therap.*, 1943, 78: 120–126.

DEARBORN, E. H., AND MARSHALL, E. K. JR. The degradation of quinine in the duck, chicken, and dog. *J. Pharmacol. & Exper. Therap.*, 1945, 85: 202–215.

HARNED, B. K., AND ETTELDORF, J. N. The concomitant administration of sulfathiazole and quinine or atabrine. *Am. J. M. Sc.*, 1944, 208: 750–756.

HARNED, B. K., MILLER, R. E., WIENER, M., AND WATTS, N. P. A potentiated chemotherapeutic action of sulfathiazole and quinine. *Proc. Soc. Exper. Biol. & Med.*, 1944, 55: 234–235.

HEGSTED, D. M., McKIBBIN, J. M., AND STARE, F. J. The effect of atabrine on choline deficiency in the young rat. *J. Nutrition*, 1944, 27: 149–153.

HEGSTED, D. M., McKIBBIN, J. M., AND STARE, F. J. The effect of atabrine on thiamin deficiency in young rats. *Federation Proc.*, 1944, 3: 94.

HEGSTED, D. M., McKIBBIN, J. M., AND STARE, F. J. The effect of atabrine on thiamine deficiency in the young rat. *J. Nutrition*, 1945, 29: 361–363.

HEGSTED, D. M., McKIBBIN, J. M., AND STARE, F. J. Nutrition and tolerance to atabrine. *J. Nutrition*, 1944, 27: 141–148.

HEWITT, R. I., AND RICHARDSON, A. P. Observation on the action of drugs in Plasmodium lophurae infections. *Federation Proc.*, 1943, 2: 82.

HEWITT, R. I., AND RICHARDSON, A. P. The direct plasmodicidal effect of quinine, atabrine and plasmochin on Plasmodium lophurae. *J. Infect. Dis.*, 1943, 73: 1–11.

HEWITT, R. I., RICHARDSON, A. P., AND SEAGER, L. D. Observations on untreated infections with Plasmodium lophurae in twelve hundred young white Pekin ducks. *Am. J. Hyg.*, 1942, 36: 362–373.

KELSEY, F. E., AND GEILING, E. M. K. The micro determination of quinine in blood and tissues. *J. Pharmacol. & Exper. Therap.*, 1942, 75: 183–186.

KELSEY, F. E., GEILING, E. M. K., OLDHAM, F. K., AND DEARBORN, E. H. Studies on antimalarial drugs. The preparation and properties of a metabolic derivative of quinine. *J. Pharmacol. & Exper. Therap.*, 1944, 80: 391–392.

KELSEY, F. E., AND OLDHAM, F. K. Studies on antimalarial drugs. The distribution of quinine oxidase in animal tissues. *J. Pharmacol. & Exper. Therap.*, 1943, 79: 77–80.

KELSEY, F. E., OLDHAM, F. K., DEARBORN, E. H., SILVERMAN, M., AND LEWIS, E. W. Studies on antimalarial drugs. The excretion of atabrine in the urine of the human subject. *J. Pharmacol. & Exper. Therap.*, 1944, *80:* 383–385.

KELSEY, F. E., OLDHAM, F. K., AND GEILING, E. M. K. Studies on antimalarial drugs. The distribution of quinine in the tissues of the fowl. *J. Pharmacol. & Exper. Therap.*, 1943, *78:* 314–319.

KELSEY, F. E., OLDHAM, F. K., AND GEILING, E. M. K. Studies on antimalarial drugs. The metabolism of quinine and quinidine in birds and mammals. *J. Pharmacol. & Exper. Therap.*, 1945, *85:* 170–175.

MARSHALL, E. K. JR., LITCHFIELD, J. T. JR., AND WHITE, H. J. The antimalarial action in ducks of certain sulfanilamide derivatives. *J. Pharmacol. & Exper. Therap.*, 1946, *86:* 273–279.

OLDHAM, F. K., AND KELSEY, F. E. Studies on antimalarial drugs. The distribution of atabrine in the tissues of the fowl and the rabbit. *J. Pharmacol. & Exper. Therap.*, 1945, *83:* 288–293.

OLDHAM, F. K., AND KELSEY, F. E. Studies on antimalarial drugs. The influence of pregnancy on the quinine oxidase of rabbit liver. *J. Pharmacol. & Exper. Therap.*, 1943, *79:* 81–84.

OLDHAM, F. K., KELSEY, F. E., CANTRELL, W., AND GEILING, E. M. K. Studies on antimalarial drugs. The effect of malaria (Plasmodium gallinaceum) and of anemia on the distribution of quinine in the tissues of the fowl. *J. Pharmacol. & Exper. Therap.*, 1944, *82:* 349–356.

SEAGER, L. D., ETTELDORF, J. N., GRENFELL, N. P., HEWITT, R. I., AND RICHARDSON, A. P. The acute toxicity of 4:4'-diamidino diphenoxy pentane and 4:4'-diamidino stilbene. *Federation Proc.*, 1943, *2:* 91.

SHANNON, J. A., EARLE, D. P. JR., BRODIE, B. B., TAGGART, J. V., AND BERLINER, R. W. The pharmacological basis for the rational use of atabrine in the treatment of malaria. *J. Pharmacol. & Exper. Therap.*, 1944, *81:* 307–330

SIEGEL, H., AND MUSHETT, C. W. Structural changes following administration of quinacrine hydrochloride. *Arch. Path.*, 1944, *38:* 63–70

SIEGEL, H., MUSHETT, C. W., AND EMERSON, G. A. Influence of protein and thiamine intake on pathologic and weight changes produced by atabrine. *Proc. Soc. Exper. Biol. & Med.*, 1945, *58:* 157–159

SILBER, R. H., CLARK, I., AND SIEGEL, H. Elevated plasma fibrinogen levels and liver necrosis in rats receiving atabrine. *Proc. Soc. Exper. Biol. & Med.*, 1944, *57:* 115–116

SILVERMAN, M., AND EVANS, E. A., JR. The effects of spermine, spermidine, and other polyamines on the growth inhibition of Escherichia coli by atabrine. *J. Biol. Chem.*, 1944, *154:* 521–534

SILVERMAN, M., AND EVANS, E. A. JR. The effects of spermidine and other polyamines on the growth inhibition of Escherichia coli by atabrine. *J. Biol. Chem.*, 1943, *150:* 265–266

SMITH, C. C., FRADKIN, R., AND LACKEY, M. D. A hemorrhagic syndrome induced by derivatives of 3-hydroxy-1, 4-naphthoquinone. *Proc. Soc. Exper. Biol. & Med.*, 1946, *61:* 398–403

TALIAFERRO, L. G., COULSTON, F., AND SILVERMAN, M. The antimalarial activity of tyrothricin against Plasmodium gallinaceum. *J. Infect. Dis.*, 1944, *75:* 179–211

THOMPSON, P. E. The effects of sulfonamide diets upon infections of Plasmodium elongatum in canaries. *J. Infect. Dis.*, 1945, *76:* 15–19

WOLFSON, F. Effect of preservation by freezing upon the virulence of Plasmodium for ducks. *Am. J. Hyg.*, 1945, *42:* 155–166

WOLFSON, F. Virulence of the IG. strain of Plasmodium relictum for the duck. *Am. J. Hyg.*, 1945, *42:* 111–118

WOLFSON, F. An experimental study of mixed infections with Plasmodium cathemerium and Plasmodium lophurae in ducks. *Am. J. Hyg.*, 1945, *41:* 123–135

Serology

KAHN, R. L., McDERMOTT, E. B., WHEELER, A. H., AND ADLER, J. Phenomenon of precipitation on mixing hemoglobin with tissue extract antigen. *Proc. Soc. Exper. Biol. & Med.*, 1945, *58:* 240–242

Synthesis of Antimalarials

BRESLOW, D. S., BAUMGARTEN, E., AND HAUSER, C. R. A new synthesis of beta-keto esters of the type $RCOCH_2COOC_2H_5$. *J. Am. Chem. Soc.*, 1944, *66:* 1286–1288

BRESLOW, D. S., AND HAUSER, C. R. The synthesis of 6-diethylamino-1-aminohexane. *J. Am. Chem. Soc.*, 1945, *67:* 686

BRESLOW, D. S., WALKER, H. G., YOST, R. S., AND HAUSER, C. R. Synthesis of quinacrine analogs having aromatic groups in the side chain. *J. Am. Chem. Soc.*, 1945, *67:* 1472–1475

BRESLOW, D. S., YOST, R. S., WALKER, H. G., AND HAUSER, C. R. Synthesis of atabrine analogs having various aliphatic alpha-substituents in the side chain. *J. Am. Chem. Soc.*, 1944, *66:* 1921–1924

BROWN, R. F., JACOBS, T. L., WINSTEIN, S., LEVY, E. F., Moss, H. R., AND OTTO, M. L. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. V. The preparation of some alpha-dialkylaminomethyl-2-chloro- and bromo-1-napthalenemethanols. *J. Org. Chem.*, 1946, *11:* 163–169.

CAMPBELL, K. N., SOMMERS, A. H., AND CAMPBELL, B. K. The preparation of t-butylamine by the low-pressure hydrogenation of 2,2-dimethylethyl-enimine. *J. Am. Chem. Soc.*, 1946, *68:* 140

DODD, T. N. JR., SCHRAMM, C. H., AND ELDERFIELD, R. C. 3-(2-diakylamino-1-hydroxyethyl) retene derivatives. *J. Org. Chem.*, 1946, *11:* 253–256

FRENCH, H. E., UNGNADE, H. E., POE, J. E., AND EILERS, L. H. Some polymethylenediamines. *J. Am. Chem. Soc.*, 1945, *67:* 882

GRIFFING, J. M., AND ELDERFIELD, R. C. 7-Methoxy-8-acetyl- and 7-methoxy-9-acetyl-1,2,3,4,-tetrahydrophenanthrene and amino carbinols derived from them. *J. Org. Chem.*, 1946, *11:* 123–135

JACOBS, T. L., WINSTEIN, S., HENDERSON, R. B., BOND, J., RALLS, J. W., SEYMOUR, D., AND FLORSHEIM, W. H. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. VIII. 5-, 6- and 7-chloro derivatives. *J. Org. Chem.*, 1946, *11:* 229–238

JACOBS, T. L., WINSTEIN, S., LINDEN, G. B., AND SEYMOUR, D. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. VII. Synthesis of some propanol-amines by means of Grignard reagents. *J. Org. Chem.*, 1946, *11*: 223–228

JACOBS, T. L., WINSTEIN, S., RALLS, J. W., AND ROBSON, J. H. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. II. 1-Halonaphthalenes in the Friedel and Crafts reaction. *J. Org. Chem.*, 1946, *11*: 27–33

JACOBS, T. L., WINSTEIN, S., RALLS, J. W., ROBSON, J. H., HENDERSON, R. B., AKAWIE, R. I., FLORSHEIM, W. H., SEYMOUR, D., AND SEIL, C. A. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. I. Amino ketone method. *J. Org. Chem.*, 1946, *11*: 21–26

KLEIMAN, M., AND WEINHOUSE, S. 6'-Methoxyrubanol-9. *J. Org. Chem.*, 1945, *10*: 562–569

KUPCHAN, S. M., AND ELDERFIELD, R. C. 7-Chloro- and 7-methoxy-8-chloro-1,2,3,4-tetrahydrophenanthrene and amino carbinols derived from them. *J. Org. Chem.*, 1946, *11*: 136–149

LUTZ, R. E., AND BAILEY, P. S. The novel reaction between amines and di-benzoylmethylethylene. Evidence for a chelate structure for cis-dibenzoyl-methylethylene. *J. Am. Chem. Soc.*, 1945, *67*: 2229–2232

MAY, E. L., AND MOSETTIG, E. Attempts to find new antimalarials. I. Amino alcohols derived from 1,2,3,4-tetrahydrophenanthrene. *J. Org. Chem.*, 1946, *11*: 1–9

MAY, E. L., AND MOSETTIG, E. Attempts to find new antimalarials. II. Amino alcohols derived from phenanthrene. *J. Org. Chem.*, 1946, *11*: 10–14

MAY, E. L., AND MOSETTIG, E. Attempts to find new antimalarials. III. Amino alcohols derived from 3-methoxyphenanthrene. *J. Org. Chem.*, 1946, *11*: 15–20

MAY, E. L., AND MOSETTIG, E. Attempts to find new antimalarials. IV. Amino alcohols derived from phenanthrene and tetrahydrophenanthrene. *J. Org. Chem.*, 1946, *11*: 105–110

McCASLAND, G. E. Derivatives of 8-chloroquinoline. *J. Am. Chem. Soc.*, 1946, *68*: 533

McCASLAND, G. E. The preparation of 8-quinolinesulfonic acid. *J. Org. Chem.*, 1946, *11*: 277–280

MEAD, F. F., AND KOEPFLI, J. B. The structure of a new metabolic derivative of quinine. *J. Biol. Chem.*, 1944, *154*: 507–515

MEAD, J. F., RAPPORT, M. M., SENEAR, A. E., MAYNARD, J. T., AND KOEPFLI, J. B. The synthesis of potential antimalarials. Derivatives of pantoyltaurine. *J. Biol. Chem.*, 1946, *163*: 465–473

MIGHTON, H. R. I. Studies on ring closure of gamma-(and 2-napthy 1)-butyric acid.

MIGHTON, H. R., AND ELDERFIELD, R. C. II. 7-Acetoxy-9-acetyl-1,2,3,4-tetrahydro-phenanthrene and dialkylamino carbinols derived from it. *Thesis privately printed by Columbia University*, 1945. Part II also in *J. Org. Chem.*, 1946, *11*: 247–252

NIGHTINGALE, D., UNGNADE, H. E., AND FRENCH, H. E. The preparation of alpha-(dialkylaminoalkyl)-acenaphthene-methanols. *J. Am. Chem. Soc.*, 1945, *67*: 1262–1264

PRICE, C. C., AND CURTIN, D. Y. 4-Hydroxypyrido (3,2-d) pyrimidine. *J. Am. Chem. Soc.,* 1946, *68:* 914

PRICE, C. C., GUTHRIE, D. B., HERBRANDSON, H. F., AND PEEL, E. W. Some decamethylene and undecamethylene diamines. *J. Org. Chem.,* 1946, *11:* 281–285

PRICE, C. C., AND HERBRANDSON, H. F. 6-Methoxy-8-(9-diethylaminononyl-amino)-quinoline dihydrobromide. *J. Am. Chem. Soc.,* 1946, *68:* 914

PRICE, C. C., AND HERBRANDSON, H. F. 6-Methoxy-8-amino-1,2,3,4-tetrahydroquinoline. *J. A. Chem. Soc.,* 1946, *68:* 910

PRICE, C. C., LEONARD, N. J., AND CURTIN, D. Y. 2-Amino-4-methyl-6-methoxy-methylpyrimidine, some derivatives and related compounds. *J. Org. Chem.,* 1945, *10:* 318–326

PRICE, C. C., LEONARD, N. J., AND REITSEMA, R. H. An isomer of thiamin. *J. Am. Chem. Soc.,* 1946, *68:* 766–769

PRICE, C. C., LEONARD, N. J., AND WHITTLE, R. L. The bromination of 2-amino-4,6-dimethylpyrimidine. *J. Org. Chem.,* 1945, *10:* 327–332

PRICE, C. C., AND STACY, G. W. p-Nitrophenyl disulfide, p-nitrophenyl sulfide and p-Nitrothiophenol. *J. Am. Chem. Soc.,* 1946, *68:* 498–500

SARGENT, L. J., AND SMALL, L. Attempts to find new antimalarials. V. Studies in the acridine series. 9-N-heterocyclic acridines and 9-acridylsulfanilamides. *J. Org. Chem.,* 1946, *11:* 175–178

SARGENT, L. J., AND SMALL, L. Attempts to find new antimalarials. VI. Some heterocyclic sulfanilamide derivatives. *J. Org. Chem.,* 1946, *11:* 179–181

TARBELL, D. S., BUNNETT, J. F., CARLIN, R. B., AND WYSTRACH, V. P. The synthesis of some 2-phenyllepidylamines. *J. Am. Chem. Soc.,* 1945, *67:* 1582–1584

UNGNADE, H. E., NIGHTINGALE, D. V., AND FRENCH, H. E. The synthesis of 7-methoxy-1-isoquinolone. *J. Org. Chem.,* 1945, *10:* 533–536

VAUGHAN, W. R. 2,4-Dihydroxy-3-quindyl methyl ketones. *J. Am. Chem. Soc.,* 1946, *68:* 324–325

WINSTEIN, S., JACOBS, T. L., HENDERSON, R. B., AND FLORSHEIM, W. H. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. III. Reduction of substituted naphthyl halomethyl ketones to halohydrins. Derived amino alcohols. *J. Org. Chem.,* 1946, *11:* 150–156

WINSTEIN, S., JACOBS, T. L., HENDERSON, R. B., ROBSON, J. H., AND DAY, B. F. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. IV. Substituted alpha-naphthylethylene oxides and derived amino alcohols. *J. Org. Chem.,* 1946, *11:* 157–162

WINSTEIN, S., JACOBS, T. L., SEYMOUR, D., AND LINDEN, G. B. Substituted alpha-dialkylaminoalkyl-1-naphthalenemethanols. VI. Some Mannich ketones and derived propanolamines. *J. Org. Chem.,* 1946, *11:* 215–222

WRIGHT, J. B., AND ELDERFIELD, R. C. 9-Alkylamino carbinols derived from 9-acyl-1,2,3,4-tetrahydrophenanthrene derivatives. *J. Org. Chem.,* 1946, *11:* 111–122

CHEMOTHERAPEUTIC AND ANTIBIOTIC AGENTS

Sulfonamide Drugs

BLISS, E. A., AND DEITZ, H. C. A comparison of the bacteriostatic activities of some of the newer sulfonamide compounds. *Bull. Johns Hopkins Hosp.*, 1944, *75:* 1–13

BLISS, E. A., AND DEITZ, H. C. The mode of action of sulfanilamide. Studies on homosulfanilamide. *J. Bact.*, 1944, *47:* 449

POSCH, J. L., MAUN, M. E., PILLING, M. A., AND HIRSCHFELD, J. W. The effect of sulfanilamide and sulfathiazole on human tissue. *Surg., Gynec., & Obst.*, 1945, *80:* 143–147

SCHMIDT, L. H., HUGHES, H. B., BADGER, E. A., AND SCHMIDT, I. G. The toxicity of sulfamerazine and sulfamethazine. *J. Pharmacol. & Exper. Therap.*, 1944, *81:* 17–42

SCHMIDT, L. H., SESLER, C. L., AND HUGHES, H. B. The chemotherapeutic activities of sulfamerazine and sulfamethazine. *J. Pharmacol. & Exper. Therap.*, 1944, *81:* 43–57

Penicillin

ANDERSON, D. G. Chemotherapy (penicillin). *Kentucky M. J.*, 1945, *43:* 99–103

ANDERSON, D. G. Clinical experience with penicillin. *New York State J. Med.*, 1944, *44:* 1651–1654

ANDERSON, D. G. Clinical experiences with penicillin. *Clin. Med.*, 1944, *51:* 145–148

ANDERSON, D. G. Clinical observations on the treatment of various infections with penicillin. *Bull. New York Acad. Med.*, 1945, *21:* 581–598

ANDERSON, D. G. The treatment of infections with penicillin. *New England J. Med.*, 1945, *232:* 400–405 and 423–429

ANDERSON, D. G. Penicillin. *Bull. New England Med. Center*, 1944, *6:* 145–152

ANDERSON, D. G. Penicillin. *Am. J. Nursing*, 1945, *45:* 18–20

ANDERSON, D. G., AND KEEFER, C. S. The treatment of staphylococcic infections with penicillin. *M. Clin. North America*, 1944, *28:* 1029–1042

ANDERSON, D. G., AND KEEFER, C. S. Treatment of infections with penicillin. Supplement to Sixth Edition Cecil's Textbook of Medicine. Russell L. Cecil's *Textbook of Med.* — 6th edition. W. B. Saunders Co., Philadelphia, 1944, pp. 1532–1536

BENEDICT, R. G., SCHMIDT, W. H., COGHILL, R. D., AND OLESON, A. P. Penicillin. III. The stability of penicillin in aqueous solution. *J. Bact.*, 1945, *49:* 85–95

ECKMAN, M., RUMSEY, C. C., JR., BARACH, B., AND BARACH, A. L. A demand apparatus for automatic delivery of aerosols during inspiration. *J. Lab. & Clin. Med.*, 1945, *30:* 608–610

GAY, L. N. The nonantigenic property of beeswax. *J. Allergy*, 1945, *16:* 192–195

KEEFER, C. S. The present status of penicillin in the treatment of infections. *Proc. Am. Philosophical Soc.*, 1944, *88:* 174–176

KEEFER, C. S. Penicillin. *Am. J. Nursing*, 1943, *43:* 1076–1077

KEEFER, C. S. Penicillin — its present status in the treatment of infections. *Am. J. M. Sc.,* 1945, *210:* 145–158

KEEFER, C. S., BLAKE, F. G., MARSHALL, E. K. JR., LOCKWOOD, J. S., AND WOOD, W. B. JR. Penicillin in the treatment of infections. A report of 500 cases. *J.A.M.A.,* 1943, *122:* 1217–1224

MCDERMOTT, W., BUNN, P. A., BENOIT, M., DuBOIS, R., AND HAYNES, W. Oral penicillin. *Science,* 1945, *101:* 228–229

MOYER, A. J., AND COGHILL, R. D. Penicillin. VIII. Production of penicillin in surface cultures. *J. Bact.,* 1946, *51:* 57–78

MOYER, A. J., AND COGHILL, R. D. Penicillin. IX. The laboratory scale production of penicillin in submerged cultures by Penicillium notatum Westling (NRRL 832). *J. Bact.,* 1946, *51:* 79–93

RAPER, K. B., ALEXANDER, D. F., AND COGHILL, R. D. Penicillin. II. Natural variation and penicillin production in Penicillium notatum and allied species. *J. Bact.,* 1944, *48:* 639–659

ROBERTS, E. C., CAIN, C. K., MUIR, R. D., REITHEL, F. J., GABY, W. L., VAN BRUGGEN, J. T., HOMAN, D. M., KATZMAN, P. A., JONES, L. R., AND DOISY, E. A. Penicillin B, an antibacterial substance from Penicillium notatum. *J. Biol. Chem.,* 1943, *147:* 47–58

SCHMIDT, W. H., AND MOYER, A. J. Penicillin. I. Methods of assay. *J. Bact.,* 1944, *47:* 199–209

VAN BRUGGEN, J. T., REITHEL, F. J., CAIN, C. K., KATZMAN, P. A., DOISY, E. A., MUIR, R. D., ROBERTS, E. C., GABY, W. L., HOMAN, D. M., AND JONES, L. R. Penicillin B: Preparation, purification, and mode of action. *J. Biol. Chem.,* 1943, *148:* 365–378

VINCENT, J. G., AND VINCENT, H. W. Filter paper disc modification of the Oxford cup penicillin determination. *Proc. Soc. Exper. Biol. & Med.,* 1944, *55:* 162–164

Other Antibiotic Agents

ANDERSON, H. H., VILLELA, G. G., HANSEN, E. L., AND REED, R. K. Some physical and biologic properties of subtilin and other antibiotics. *Science,* 1946, *103:* 419–420

GABY, W. L. A study of the dissociative behavior of Pseudomonas aeruginosa. *J. Bact.,* 1946, *51:* 217–234

HAYS, E. E., WELLS, I. C., KATZMAN, P. A., CAIN, C. K., JACOBS, F. A., THAYER, S. A., DOISY, E. A., GABY, W. L., ROBERTS, E. C., MUIR, R. D., CARROLL, C. J., JONES, L. R., AND WADE, N. J. Antibiotic substances produced by Pseudomonas aeruginosa. *J. Biol. Chem.,* 1945, *159:* 725–750

JOHNSON, B. A., ANKER, H., AND MELENEY, F. L. Bacitracin: A new antibiotic produced by a member of the B. subtilis group. *Science,* 1945, *102:* 376–377

KATZMAN, P. A., HAYS, E. E., CAIN, C. K., VAN WYKE, J. J., REITHEL, F. J., THAYER, S. A., DOISY, E. A., GABY, W. L., CARROLL, C. J., MUIR, R. D., JONES, L. R., AND WADE, N. J. Clavacin, an antibiotic substance from Aspergillus clavatus. *J. Biol. Chem.,* 1944, *154:* 475–486

LEOPOLD, I. H., AND NICHOLS, A. Intraocular penetration of streptomycin following systemic and local administration. *Arch. Ophthal.,* 1946, *35:* 33–38

MOLITOR, H., GRAESSLE, O. E., KUNA, S., MUSHETT, C. W., AND SILBER, R. H. Some toxicological and pharmacological properties of streptomycin. *J. Pharmacol. & Exper. Therap.*, 1946, *86:* 151–173

WOLTZ, J. H. E., AND WILEY, M. M. Transmission of streptomycin from maternal blood to the fetal circulation and the amniotic fluid. *Proc. Soc. Exper. Biol. & Med.*, 1945, *60:* 106–107

ZINTEL, H. A., FLIPPIN, H. F., NICHOLS, A. C., WILEY, M. M., AND RHOADS, J. E. Studies on streptomycin in man. I. Absorption, distribution, excretion, and toxicity. *Am. J. M. Sc.*, 1945, *210:* 421–430

LIST OF CONTRACTS
OSRD Medical Research Contracts
Sponsored by the
Committee on Medical Research

DIVISION OF MEDICINE

Infectious Diseases

Contract OEMcmr-	Contractor	Investigator	Subject
18	University of Kentucky	P. R. Edwards	Preparation of diagnostic sera for the identification and classification of typhoid and paratyphoid infections.
31	New York University	Mark H. Adams Alan W. Bernheimer Alwin M. Pappenheimer	Preparation of *Clostridium welchii* toxoid for active immunization against gas gangrene and *Cl. septicum* toxin, and study of the value of septicum antitoxins.
35	University of Cincinnati	Milan A. Logan	Development of an effective toxoid from *Cl. welchii* toxin.
45	University of California	K. F. Meyer H. Sommer E. E. Baker	Studies in connection with plague vaccine, immunochemistry of the plague bacillus, and chemoserum therapy of plague.
48	University of Rochester	Andrew H. Dowdy	Effect of sulfonamide drugs upon experimental Clostridium infection in dogs.
75	Harvard University	J. Howard Mueller	Preparation of tetanus toxoid.
117	Rockefeller Institute	Frank L. Horsfall Oswald T. Avery	Primary atypical pneumonia (acute pneumonitis).
118	Rockefeller Institute	Homer F. Swift	Identification and designation by numbers of types of group A hemolytic streptococci not included in Griffith's types 1–30, and comparison of typing strains of group A hemolytic streptococci by anti-M precipitation and slide agglutination technics.

Contract OEMcmr-	Contractor	Investigator	Subject
120	University of Pennsylvania	Stuart Mudd	Production of a dysentery vaccine of low toxicity.
136	University of Chicago	Benjamin F. Miller	Germicidal properties of synthetic detergents.
138	University of California	W. McD. Hammon	The role of arthropods in the transmission of encephalitis.
140	Biochemical Research Foundation	R. W. Linton R. K. Jennings	Cholera vaccine.
150	University of Chicago	G. M. Dack	Chemotherapy in bacillary dysentery, treatment of the disease, and especially for clearing of the carrier state.
154	University of Chicago	William Burrows	Active immunization against Asiatic cholera.
158	Rockefeller Institute	W. M. Stanley	Influenza virus and Japanese B encephalitis vaccines.
161	House of Good Samaritan	T. Duckett Jones	An attempt to control the spread of hemolytic streptococcus infection at the United States Naval Training Station, Newport, Rhode Island.
170	Harvard University	René J. Dubos Henry P. Treffers	Immunochemical investigations directed toward active immunization and production of therapeutic agents against bacillary dysentery.
175	Northwestern University	B. H. Jennings Edward Bigg	Ventilating problems involved in the practical utilization of propylene glycol and other glycol vapors as means of air sterilization for the control of air-borne infections.
216	Rockefeller Institute	Walther F. Goebel	Antigenic constituents of dysentery bacilli with special reference to the Flexner group; such studies to aid development of a satisfactory vaccine for human use.
228	Columbia University	Erwin Chargaff	Purification of typhus vaccine and the chemical nature of rickettsia antigens.

Contract OEMcmr-	Contractor	Investigator	Subject
229	Stanford University	Lowell A. Rantz	Relation of immunologic phenomena in hemolytic streptococcus infection to the clinical course of the disease and the development of complications.
259	University of California	K. F. Meyer H. Sommer E. E. Baker	Plague vaccine.
293	Children's Hospital, Cincinnati	Merlin L. Cooper B. K. Rachford A. A. Weech	Immunization against bacillary dysentery.
308	University of Southern California	Frederick J. Moore John F. Kessel	Sulfonamide therapy of bacillary dysentery and classification of etiologic agents encountered.
329	University of Pennsylvania	Leslie A. Chambers Thomas F. Anderson	Improvement of the immunization capacity of typhus vaccines.
360	Children's Hospital, Philadelphia	Werner Henle	Interference of inactive influenza virus with the propagation of the active agent in the chick embryo, mouse, and man.
382	University of Chicago	O. H. Robertson	Factors in dissemination of pathogenic bacteria and viruses in the air and methods of control of air-borne infections.
383	Michael Reese Research Foundation	Sidney O. Levinson	Development of an ultraviolet lamp for inactivation of bacterial and viral suspensions.
418	University of Colorado	James J. Waring	The course and prognosis of minimal pulmonary tuberculosis in men in the military service and the determination of the influence of physical strain and emotional factors upon tuberculosis.
422	Columbia University	Donovan J. McCune Hattie E. Alexander	Development of antitoxin in the serum of human volunteers following injections of perfringens toxoid.
438	Children's Hospital, Philadelphia	Joseph Stokes, Jr.	Methods of control of airborne infections.

Contract OEMcmr-	Contractor	Investigator	Subject
444	Sharp and Dohme		Manufacture of influenza vaccine.
449	Rockefeller Institute	René J. Dubos	Bacillary dysentery.
460	Washington University	Robert A. Moore	Effect of penicillin on the character and severity of bacterial infections in man.
464	George Washington University	Harry F. Dowling	Immunization against gas gangrene.
470	Edward J. Meyer Memorial Hospital	David K. Miller	Antibacterial activity of molds against tubercle bacilli and tuberculosis.
472	E. R. Squibb and Sons		Manufacture of influenza vaccine.
479	Mt. Sinai Hospital	George Baehr Gregory Shwartzman I. E. Gerber	Penicillin therapy of subacute bacterial endocarditis due to *Streptococcus viridans*, enterococcus, and other organisms.
489	University of Pittsburgh	Max A. Lauffer	Influenza virus.
499	Yale University	Henry P. Treffers	Immunochemical investigations directed toward active immunization and production of therapeutic agents against bacillary dysentery.
520	University of California	William J. Kerr	Penicillin treatment of subacute bacterial endocarditis and combined penicillin-sulfadiazine treatment of pneumococcic meningitis, and prolongation of absorption of penicillin by various vehicles and by sublingual administration.
521	Lederle Laboratories		Manufacture of anti-plague serum.
541	Carnegie Institution of Washington	M. Demerec	Genetic aspects of the origin of bacterial resistance to various drugs.
568	E. R. Squibb and Sons		Manufacture of anti-plague serum.
M–698	National Institute of Health	Albert V. Hardy James Watt	Sulfaguanidine in the enteric infections with chief attention to the control of *Shigella dysenteriae* infections.

Contract OEMcmr-	Contractor	Investigator	Subject
M–834	National Institute of Health	Eloise B. Cram	Effect of various treatment processes on the survival of helminth ova and protozoan cysts in sewage.

Venereal Diseases

53	Harvard University	J. Howard Mueller	Growth requirements of the gonococcus.
61	University of Georgia	Robert B. Greenblatt	Diagnosis, treatment, and prevention of the newer venereal diseases.
84	Southwestern Medical Foundation	Arthur G. Schoch Lee J. Alexander	Treatment of syphilis.
99	University of Rochester	Charles M. Carpenter Stafford L. Warren	Chemotherapy of syphilis and other venereal diseases.
137	New York University	Frank C. Combes	Diagnosis of lymphogranuloma venereum and the prophylaxis of chancroid.
181	Rockefeller Institute	Frederik B. Bang	Chemical prophylaxis of gonorrhea.
197	Johns Hopkins University	Justina H. Hill	Establishment of gonococcal infection in experimental animals by methods applicable to the study of venereal disease.
202	Western Reserve University	Herbert Lund	Nature of biologic false-positive reactions in serology of syphilis.
204	Warner Institute	Marvin R. Thompson	Chemoprophylaxis in gonorrhea, venereal lymphogranuloma, and chancroid, and sulfonamides in ointment bases.
207	E. R. Squibb and Sons	Geoffrey W. Rake	Prophylaxis against lymphogranuloma venereum, and the development of a prophylactic agent effective against all venereal diseases.
213	Western Reserve University	John Wellman B. S. Kline H. P. Lankelma	Isolation of the most potent and most specific fraction of tissue extracts (beef-heart powder) for use in diagnostic tests for syphilis.
215	Johns Hopkins University	Harry Eagle	Treatment and prophylaxis of venereal diseases.

Contract OEMcmr-	Contractor	Investigator	Subject
254	Columbia University	Dan H. Moore Elvin A. Kabat	Biologic false-positive serologic tests for syphilis.
255	Duke University	Joseph W. Beard Hans Neurath	Biologic false-positive serologic tests for syphilis.
316	University of Chicago	C. Philip Miller	Evaluation of prophylactics for venereal diseases.
325	University of North Carolina	William L. Fleming	Relative prophylactic effectiveness against syphilis of ointments containing calomel in different particle size, and the treatment of syphilis.
331	Johns Hopkins University	Alan M. Chesney	Local prophylaxis against syphilis.
349	University of Rochester	Charles M. Carpenter Stafford L. Warren	Effect of heavy-metal inunctions on the prophylaxis of experimental syphilis in rabbits, and influence of the vehicle in calomel ointment on the efficacy of the ointment as a prophylactic agent.
374	Johns Hopkins University	J. Earle Moore Harry Eagle	Serologic pattern of syphilitic and nonsyphilitic human sera and animal sera.
393	Johns Hopkins University	J. Earle Moore Charles F. Mohr Russell A. Nelson Harold A. Tucker	Effect of penicillin in syphilis, especially in late syphilis.
401	University of Pennsylvania	John H. Stokes Fred Boerner A. P. Hitchens	Biologic false-positive serologic tests for syphilis following donation of blood.
403	University of Pennsylvania	John H. Stokes	Effect of penicillin on certain aspects of syphilis.
404	New York University	Evan W. Thomas Bernhard Dattner	Treatment of early syphilis with penicillin.
435	Cornell University	Walsh McDermott	Effectiveness of penicillin therapy in syphilis.
446	Duke University	J. Lamar Callaway	Penicillin treatment for syphilis.
461	Tulane University	R. V. Platou	Treatment of congenital syphilis with penicillin.
480	Wills Hospital, Philadelphia	Joseph V. Klauder	Penicillin in treatment of ocular syphilis.

Contract OEMcmr-	Contractor	Investigator	Subject
495	University of Virginia	Dudley C. Smith	Penicillin in the treatment of syphilis.
496	University of Texas	Chester N. Frazier	Use of penicillin in the treatment of syphilis, acquired and congenital.
497	Western Reserve University	Harold N. Cole	Penicillin therapy of syphilis.
505	Vanderbilt University	R. H. Kampmeier	Effectiveness of penicillin in the treatment of syphilis.
510	Chicago Board of Health	Herman N. Bundesen	Penicillin in venereal disease (syphilis).
511	Wayne University	Loren W. Shaffer	Penicillin therapy of syphilis.
514	Stanford University	Charles W. Barnett	Treatment of early syphilis with penicillin.
515	University of Michigan	Udo J. Wile A. C. Curtis	Penicillin therapy for early and late syphilis.
519	Washington University	E. Gurney Clark	Treatment of early syphilis with penicillin.
526	Johns Hopkins University	Alan M. Chesney	Efficacy of derivatives from commercial penicillin in the treatment of experimental syphilis in the rabbit.
527	Johns Hopkins University	Edwin L. Crosby N. A. Nelson	Biostatistical analysis of nation wide results of penicillin therapy in syphilis.
543	New York University	Franco Mortara	Diagnosis, treatment, and epidemiology of chancroid.
558	New Britain General Hospital	Paul D. Rosahn	Penicillin and its fractions and other antibiotic agents in experimental rabbit syphilis.
M-1997	Food and Drug Administration, Federal Security Agency	Herbert O. Calvery	Pharmacologic and toxicologic investigation of chemical prophylactics for venereal diseases.
M-3169	United States Public Health Service	J. F. Mahoney	Prevention of gonococcal infection in experimentally infected human male volunteers.
M-5961	United States Public Health Service	Harry Eagle	Penicillin therapy of experimental syphilis.

Tropical Diseases and Mycotic Infections

Contract OEMcmr-	Contractor	Investigator	Subject
105	University of Maryland	Lawrence H. James	Sterilization of used clothing, shoes, etc., by the steam vapor and ethylene oxide gas processes.
190	Columbia University	J. Gardner Hopkins	Treatment and prophylaxis of dermatophytosis.
295	Johns Hopkins University	Edmund L. Keeney	Effectiveness of sodium propionate and other agents in the treatment of cutaneous and deep fungus infections.
369	New York University	Horace W. Stunkard	Possible snail intermediate hosts of schistosomes (blood flukes) of man in the United States.
400	Tulane University	Ernest Carroll Faust Ralph G. Smith	Toxicologic and therapeutic action of certain drugs for possible use in treatment of schistosomiasis.
447	Merck Institute	Harry J. Robinson	Development of standardized methods for the rapid in vitro and in vivo testing of antifilarial agents.
455	Rice Institute	Asa C. Chandler	Ability of *Phlebotomus diabolicus* to transmit leishmaniasis.
456	University of California	H. H. Anderson Gordon A. Alles	Chemotherapy of amebiasis and of leishmaniasis, using established technics for the evaluation of antiparasitic agents.
463	Johns Hopkins University	Gilbert Fred Otto	Chemotherapy of tropical diseases.
466	Columbia University	H. B. van Dyke Alfred Gellhorn	Chemotherapy of leishmaniasis and filariasis and the pharmacology of drugs found to be useful in the treatment of these diseases.
468	Western Reserve University	Arnold D. Welch	Pharmacologic studies of agents under investigation for therapeutic application in filariasis, leishmaniasis, and schistosomiasis.
469	New York University	Arthur C. DeGraff	Chemotherapy of schistosomiasis.

Contract OEMcmr-	Contractor	Investigator	Subject
477	University of Minnesota	Raymond N. Bieter	Pharmacology of those drugs found to have chemotherapeutic activity in filariasis, leishmaniasis, and schistosomiasis, and chemotherapy of experimental filariasis.
490	Columbia University	James R. Culbertson Harry M. Rose	Chemotherapy of tropical diseases, with particular reference to schistosomiasis and filariasis.
528	Northwestern University	W. H. Abbitt	Quantitative determination of antimony and other metals in physiological fluids and tissues.

Convalescence, Neuropsychiatry, and Miscellaneous Medical Studies

Contract OEMcmr-	Contractor	Investigator	Subject
17	University of Colorado	Franklin G. Ebaugh Jack R. Ewalt	Association-motor studies in military psychiatry.
65	University of Colorado	Franklin G. Ebaugh Edward G. Billings	Analysis of 100 recent psychiatric casualties in the 8th Corps area, after induction into the Army, for the purpose of improving the diagnosis of personality disorders of recruits and selectees.
157	Massachusetts General Hospital	Paul D. White Stanley Cobb	A disorder variously termed neurocirculatory asthenia, DaCosta's syndrome, soldier's heart, or effort syndrome.
169	Massachusetts General Hospital	Fuller Albright	Metabolic studies in connection with convalescence.
177	University of Chicago	Allan T. Kenyon Wright R. Adams Ward C. Halstead	Metabolic, neuropsychological, and circulatory aspects of convalescence.
189	Johns Hopkins University	John E. Howard	Fracture healing and associated metabolic disturbances.
205	Massachusetts General Hospital	Chester M. Jones	Alleviation of pain (elevation of pain-sensitivity threshold) by monoacetyl morphine as compared with morphine sulfate administered alone or with prostigmine bromide.

Contract OEMcmr-	Contractor	Investigator	Subject
211	Cornell University	Harold G. Wolff	Development of methods for assaying the neurotic potentialities of the individual casualty for the purpose of ascertaining the management of convalescence and rehabilitation and estimating prognosis.
231	University of Michigan	F. A. Coller M. H. Seevers	Clinical evaluation of new pain-relieving drugs.
270	University of Pennsylvania	W. D. Stroud	Re-examination of registrants rejected for cardiovascular defects.
272	Columbia University	Robert L. Levy	Studies on blood pressure in Army officers.
326	New York University	Frank W. Co Tui Arthur M. Wright	Surgical and clinical nutrition.
337	Yale University	Clements C. Fry	Certain psychiatric problems of wartime medical administration and of war medicine.
362	Johns Hopkins University	L. Emmett Holt	Human amino acid requirements.
386	Cornell University	Thomas A. C. Rennie	Psychiatric rehabilitation of discharged servicemen.
402	Long Island College of Medicine	George B. Ray J. Raymond Johnson	Correlation of reduction time of the blood with physical fitness during recovery from traumatic injury, acute disease, and "war neuroses."
407	Vanderbilt University	George R. Meneely Paul F. Hahn	Alterations of cardiovascular dynamics in the convalescent state and physiological measurements correlated with clinical observations.
413	University of Minnesota	Ancel Keys	Nutrition, training, and disuse factors in physical deterioration and rehabilitation.
417	Cornell University	David P. Barr	Effect of long-continued bed rest on certain vital functions of a normal man.
420	Yale University	John P. Peters	Prevention of protein wastage in injury and disease.
436	University of Pennsylvania	Jonathan E. Rhoads Isaac Starr	Convalescence in surgical patients.

Contract OEMcmr-	Contractor	Investigator	Subject
448	University Hospitals of Cleveland	Reginald A. Shipley	Adrenocortical function during recovery from infection and from trauma as measured by assay of cortin-like material in the urine.
451	University of California	Karl M. Bowman	Problems related to protracted convalescence from injury and disease.
452	Columbia University	Sidney C. Werner	Use of amino acid mixtures as a supplement to the diet in the prevention of wasting.
454	Johns Hopkins University	L. Emmett Holt	Amino acid metabolism in disease.
478	Massachusetts General Hospital	Allan M. Butler	Efficient provision to patients subsisting on parenteral fluids of calories, nitrogen, and electrolytes to accomplish the minimal loss of body fluid.
484	University of Illinois	W. H. Cole R. W. Keeton H. H. Mitchell N. O. Callaway	Development of methods of estimating speed of postoperative convalescence, and role of ambulation and increased caloric intake in recovery.
500	New York University	L. Emmett Holt	Amino acid metabolism in disease.
502	University of Utah	Maxwell M. Wintrobe Philip B. Price	Nature, cause, and treatment of the anemia associated with infections and burns.
508	Cornell University	Harold G. Wolff	Changes in cardiovascular functions associated with emotions, acute diseases, and the convalescent state.
513	Stanford University	Ernest R. Hilgard Tamara Dembo	Social-psychological rehabilitation of the physically handicapped.

DIVISION OF SURGERY
Wounds and Burns

Contract OEMcmr-	Contractor	Investigator	Subject
37	Wayne University	John W. Hirshfeld Charles G. Johnston C. Fremont Vale William E. Abbott Frieda L. Meyer	Contaminated wounds and burns.
41	Massachusetts General Hospital	Champ Lyons	Clinical and bacteriologic study of contaminated wounds and methods of treatment with chemotherapeutic agents.
50	Tulane University	Alton Ochsner	Clinical and bacteriologic study of contaminated wounds and methods of treatment with the newer chemotherapeutic agents.
56	University of Pennsylvania	John S. Lockwood Jonathan E. Rhoads	Clinical and bacteriologic study of contaminated wounds and method of treatment with the newer chemotherapeutic agents.
62	University of Cincinnati	William A. Altemeier Max M. Zinninger Mont R. Reid	Clinical and bacteriologic study of contaminated wounds and methods of treatment with the newer chemotherapeutic agents, also study of the treatment of established infections.
78	Johns Hopkins University	Perrin H. Long Eleanor A. Bliss Warfield M. Firor Russell A. Nelson	Chemoprophylaxis and chemotherapy of wounds and burns.
79	Columbia University	Frank L. Meleney Ivan C. Hall	Classification of organisms derived from infected wounds.
80	Columbia University	Frank L. Meleney Robert Elliott Jr. Frederick Smith	Contaminated wounds and methods of treatment with the newer chemotherapeutic agents.
85	Presbyterian Hospital	Frank L. Meleney	Statistical summary of data obtained from institutions or individuals designated by the Government and engaged in the study of contaminated wounds.

Contract OEMcmr-	Contractor	Investigator	Subject
86	Presbyterian Hospital	Frank L. Meleney	Clinical and bacteriologic study of contaminated wounds and methods of treatment with the newer chemotherapeutic agents.
102	Harvard University	Oliver Cope	Effect of chemical agents on the healing of burns.
123	Henry Ford Hospital	Roy D. McClure Conrad R. Lam Frank W. Hartman	Burns, clinical and experimental.
149	Columbia University	Edward L. Howes Henry S. Simms	Regeneration of tissues during wound healing.
164	Harvard University	Valy Menkin	Chemical factors concerned in promoting proliferation of cells or repair of wounds, as manifested in an inflamed area.
176	Northwestern University	Sumner L. Koch	Clinical and bacteriologic study of burns.
178	University of Chicago	Lester R. Dragstedt H. P. Jenkins	Local treatment of burns.
183	Johns Hopkins University	Isidore Gersh	Development of rapidly acting, painless, nontoxic, membrane-forming agents acting superficially for use in treating large burns.
195	University of California	Alfred Marshak	Wound healing — methods for its determination and factors which influence it.
230	Columbia University	Charles L. Fox Jr.	Use of soluble derivatives of sulfadiazine and sulfathiazole and other therapeutic procedures in the treatment of wounds and burns.
263	Harvard University	Charles C. Lund F. H. L. Taylor	Treatment of human burns.
280	Pennsylvania Hospital	Walter Estell Lee	Status of liver function following open methods of treatment as compared with liver function following tannic acid and other closed methods.
281	Scripps Memorial Hospital	Eaton M. MacKay	Effect of urea in combination with sulfonamide drugs in relation to the treatment of wounds.

Contract OEMcmr-	Contractor	Investigator	Subject
294	University of Minnesota	William G. Clark N. Logan Leven H. M. Tsuchiya E. A. Strakosch Milton Levine	Peroxide-sulfonamide therapy of infected wounds, especially gangrene; chemical and bacteriologic studies of substances which antagonize sulfonamide inhibitors and resistance and enhance sulfonamide bacteriostasis; and chemical, animal, and clinical studies of a new burn treatment.
300	Johns Hopkins University	Warfield M. Firor Eleanor A. Bliss	Control of wound infections.
307	Harvard University	Oliver Cope Richard H. Sweet	Bacterial, chemotherapeutic, and wound-healing study of burns.
311	Duke University	Roger D. Baker	Tannic acid and burns.
322	Harvard University	Henry K. Beecher Otto Krayer Gordon K. Moe (Cancelled — no work was done)	Pulmonary effect of burns (heat and irritant gases).
334	Columbia University	Frank L. Meleney	Vehicles and adjuvants for sulfonamides for local bacteriostasis in war wounds and burns.
335	Columbia University	Sidney C. Werner	Urinary 17-ketosteroid excretion in burns as a method of prognosis and as an index of pituitary failure, and the effect of methyl testosterone in causing nitrogen retention in burn cases, and to note the effect of such treatment on the course.
336	Columbia University	George K. Smelser	Effect of anesthetics, ointments, and antiseptics on cellular proliferation and migration in the corneal epithelium following burns and other injuries.
347	Duke University	Roger D. Baker	Substances used in the treatment of burns and the pathology of burns in the human.

Contract OEMcmr-	Contractor	Investigator	Subject
359	University of Buffalo (Cancelled before work was begun)	John D. Stewart	Penicillin therapy of war wounds.
367	University of California	I. L. Chaikoff	Isolation and identification of the toxic factor in the tissues and fluids of the burned animal, and methods designed to protect the animal from deleterious effects of burn toxin.
371	Washington University	Robert Elman	Human burns.
378	University of Minnesota	W. D. Armstrong	Quinones and related substances as therapeutic agents in localized infections.
426	University of Illinois	John T. Reynolds	Value of penicillin in the treatment of compound fractures.
427	Tufts College	Otto J. Hermann	Treatment of compound fractures with prophylactic penicillin therapy.
431	University of California	Howard C. Naffziger LeRoy C. Abbott Horace McCorkle	Use of penicillin in infections of the nervous system, pleura, and bone, with special reference to craniocerebral infection.
432	Wayne University	John W. Hirshfeld Arthur H. Smith	Protein metabolism of burned dogs, and the effect upon it of amino acids and/or testosterone administered parenterally.
441	Wayne University	John W. Hirshfeld	Electrolyte and water distribution in the burn patient, with special reference to the influence of oral administration of sodium lactate solution.
457	Hospital for Joint Diseases	Joseph Buchman John E. Blair	Use of penicillin in the therapy of acute and chronic hematogenous osteomyelitis; resistance of staphylococci to penicillin; and the relation of resistance to the biology of the staphylococcus.

Contract OEMcmr-	Contractor	Investigator	Subject
462	Vanderbilt University	Cobb Pilcher J. Cyril Peterson	Clinical value of penicillin in various infectious conditions.
473	Harvard University	F. H. L. Taylor	Abnormalities in metabolism of body constituents and nutrition in burns and in certain medical and surgical conditions.
517	Tulane University	Guy A. Caldwell	Clinical evaluation of surgical technics designed to secure union in chronically infected compound fractures.
518	University of Cincinnati	W. A. Altemeier	Nature and control of Clostridial wound infections.
534	Southern Research Institute	Wilbur A. Lazier	Chemical débridement of burns.

Neurosurgery

34	Northwestern University	Loyal Davis Lewis J. Pollock	Methods of nerve suture.
68	Vanderbilt University	Sam L. Clark James W. Ward	Influence of experimental concussion on cerebral physiology.
76	Vanderbilt University	Cobb Pilcher William F. Meacham	Chemotherapy of intracranial wounds.
81	Cornell University	Joseph C. Hinsey	Nerve regeneration in relation to muscle function.
87	Jewish Hospital of Brooklyn	I. M. Tarlov Leo M. Davidoff	Autologous and homologous plasma clot and silk suture of peripheral nerves in monkeys and dogs.
88	Northwestern University	Lewis J. Pollock	Effect of physiotherapy on restoration of form and function of muscles after denervation and suture of nerves.
93	University of California	J. B. de C. M. Saunders Webb Haymaker	Effects of sulfonamide compounds on nerve tissue.
109	University of California	Robert B. Aird Howard C. Naffziger	Relative capacities of regeneration of nerve and muscle with the object of evaluating the factors responsible for the faulty recovery of the neuromuscular mechanism in man.
126	University of California	Karl M. Bowman Howard C. Naffziger	Post-traumatic personality.

Contract OEMcmr-	Contractor	Investigator	Subject
148	Columbia University	Tracy J. Putnam	Evaluation of psychological and neurologic factors in late effects of head injury.
156	University of Oregon	Robert S. Dow John E. Raaf	Relation between concussion and electroencephalographic changes in experimental animals, and the electroencephalographic changes in humans in acute cases of concussion.
159	Harvard University	Derek E. Denny-Brown Donald Munro	Analysis of the post-traumatic cerebral syndrome in relation to the type and pathology of the injury, the pre-traumatic personality, and emotional stress at the time of the injury.
160	Harvard University	Derek E. Denny-Brown	Nature of nerve lesions induced by continued pressure on nerve and its relationship to ischemic lesion in nerve.
221	University of Chicago	Paul A. Weiss	Nerve regeneration.
285	University of Chicago	A. Earl Walker Jerry J. Kollros	Acute and chronic neurologic, neuropsychological and neurophysiological effects of head injuries.
302	University of Texas	Joseph Thomas Roberts	Role of the blood supply of peripheral nerves, particularly in lesions of the peripheral nerve due to pressure, stretching, crushing, tourniquets, and other injuries common in war.
348	Northwestern University	William F. Windle	Alterations in activity and intrinsic structure of the central nervous system in experimental cerebral concussion.
355	Harvard University	Derek E. Denny-Brown	Pathology and pathophysiology of nerve injuries induced by exposure to cold.
415	Washington University	Joseph Erlanger	Waning and waxing of electrical activity, excitability, conductivity, etc., in degenerating and regenerating nerve fibers.

Contract OEMcmr-	Contractor	Investigator	Subject
437	Northwestern University	William F. Windle	Correlation between neuropathologic and psychophysiological changes after experimental concussion.
485	Cornell University	Harold G. Wolff	Post-traumatic headache.
487	Northwestern University	William F. Windle	Reaction of the spinal cord to injury.
491	University of Pennsylvania	Martin G. Larrabee Robert Hodes	Applications of electrophysiological technics to the diagnosis of peripheral nerve injury and regeneration.
492	Johns Hopkins University	Curt P. Richter	Peripheral nerve injuries.
493	Columbia University	Tracy J. Putnam	Assessment of the value of plasma clot repair of nerves.
501	University of Chicago	A. Earl Walker Herbert C. Johnson	Effects of penicillin therapy on the central nervous system.
504	Northwestern University	Lewis J. Pollock	Electrodiagnosis.

Surgical Specialties

63	Columbia University	Colin G. Fink Clay R. Murray	Determination of optimum metal for internal fixation of fractures, of optimum form and method of manufacture, and of optimum technic of internal fixation.
89	Washington University	J. Albert Key	Effect of various forms of treatment on paralyzed muscles.
94	University of California	LeRoy C. Abbott J. B. de C. M. Saunders	Promotion of union in ununited fractures.
116	University of Chicago	William E. Adams	Use of plasma for filling the pleural space after loss of varying amounts of lung.
171	University of Minnesota	Wallace D. Armstrong	Fracture calcification and healing as influenced by parenteral administration of glycerophosphate and other procedures, including the administration of testosterone propionate.

Contract OEMcmr-	Contractor	Investigator	Subject
185	University of Pennsylvania	Eugene P. Pendergrass Robert H. Ivy	Prevention of the growth of hair on pedicle flaps and skin grafts used for repair of surface defects.
198	Cornell University	Arthur H. Blakemore Bronson Ray	Development of a non-suture method of anastomosing severed arteries.
214	Harvard University	Elliott C. Cutler James B. Blodgett	Skeletal attachment of prostheses for the leg.
243	University of Chicago	Paul C. Hodges	Photoelectric x-ray exposure meters and timing devices.
265	Elizabeth Gamble Deaconess Home operating Christ Hospital, Cincinnati	Leon H. Schmidt	Pharmacologic and toxicologic investigations of monomethyl and dimethyl sulfadiazine.
283	Washington University	Peter Heinbecker Jacques Bruneau	Effects of reduced temperatures on experimentally produced infections.
286	Massachusetts Institute of Technology	Francis O. Schmitt	Development of preparations for the repair of surgical and military wounds and burns.
287	New York University	Margaret M. Hoskins	Rate of healing of bone fractures.
318	University of Georgia	Robert A. Woodbury	Influence of anesthesia, oxygen therapy, and different forms of artificial respiration on the pulmonary and systemic blood pressure in normal animals and in those with pulmonary edema.
363	Elizabeth Gamble Deaconess Home operating Christ Hospital, Cincinnati	Leon H. Schmidt	Antibacterial activity of sulfonamides synthesized as antimalarials.
370	Washington University	Peter Heinbecker Alfred Large	Influence of tissue cooling on wound healing.
388	Columbia University	Virginia Kneeland Frantz	Absorbent pledgets, membranes, and similar materials in surgical procedure.

Contract OEMcmr-	Contractor	Investigator	Subject
395	Princeton University	E. Newton Harvey Elmer G. Butler	Quantitative study of magnitude of wound produced in tissues of various densities, elasticities, rigidities, and mixed structure, by fragments of different mass, velocity, shape, direction of hit (in different planes), and angle of hit (between surface of body and surface of fragment), together with the protective effect of clothing and armor.
424	University of Rochester	Richard F. Riley	Effect of choline on the metabolism of bone, with special reference to fracture repair.
433	American Viscose Corporation		Production of collagen sutures.
476	Stanford University	J. M. Crismon John Field, 2nd	Quantitative application of combined low temperature and pulsating external pressure in the prevention of gangrene associated with massive local edema in immersion foot, frostbite, burns, and other injuries.
494	University of Cincinnati	Albert L. Brown	Use of fibrin as a mechanical adhesive in ophthalmic surgery.
512	Yale University	Samuel C. Harvey John Howard Kay	Peritonitis.
522	National Academy of Sciences	Paul E. Klopsteg	Prosthetic devices.
525	University of Pennsylvania	Francis Heed Adler	Determination of the value of preserved corneal tissue (Weiss method) for corneal transplantation.
533	New York Medical College	Kurt Lange Linn J. Boyd	Prevention of gangrene subsequent to frostbite and immersion foot.
549	Yale University	Harold Lamport Gervase Connor	Wound ballistics.

Contract OEMcmr-	Contractor	Investigator	Subject
550	Tulane University	George E. Burch	Trench foot.
M–624	Army Medical Center	Roger G. Miller Don H. Cash	Materials and processes for artificial replacement of mutilated parts of the body, especially the face and jaws, in cases incapable of satisfactory surgical repair.

DIVISION OF AVIATION MEDICINE

14	University of Pennsylvania	F. H. Lewey Donald Scott	Effects of mild oxygen deficiencies and low carbon monoxide concentration in animals and in man.
19	Yale University	John F. Fulton	Compilation of a classified bibliography of aviation medicine.
20	University of Virginia	Eugene M. Landis S. W. Britton	Methods to protect aviators against "G" (excessive gravitational and acceleratory forces in dive-bombing maneuvers).
25	Johns Hopkins University	E. Cowles Andrus	Testing of pneumatic leggings in acceleration.
26	University of Pennsylvania	Detlev W. Bronk G. A. Millikan A. J. Rawson	Improvement and testing of oxygen supply systems of military airplanes and associated physiological problems.
28	University of Pennsylvania	Detlev W. Bronk Carl F. Schmidt A. J. Rawson	Effects of low oxygen and low temperature on respiratory, cardiovascular, and visual functions and on muscular activity, and investigations in connection with the development of new instruments for testing and improving human performance at high altitudes.
30	Harvard University	Stanley Cobb L. Raymond Morrison	Effect of anoxia on nerve cells as related to the oxygen content of the blood.

Contract OEMcmr-	Contractor	Investigator	Subject
36	University of Cincinnati	M. A. Blankenhorn Eugene B. Ferris, Jr.	Nature and cause of cerebral and circulatory abnormalities in human beings, other than those due to arterial O_2 unsaturation, which occur during exposure to low atmospheric pressure, and problems related to aviation medicine.
38	Yale University	John F. Fulton	Effects of anoxia and of decompression in man and animals.
43	University of Minnesota	Gerald T. Evans	The adrenal cortex in anoxia and exposure.
47	Columbia University	Alvan L. Barach Walter W. Palmer	Testing of oxygen equipment and the equipment of a chamber with refrigeration, and a study of the effect of drugs on altitude tolerance.
60	Johns Hopkins University	George W. Thorn	The role of the adrenal cortex in anoxia.
64	University of Colorado	R. G. Gustavson	Relationship of sterols to resistance to anoxia.
74	Ohio State University	Fred A. Hitchcock Frank A. Hartman	Physiological factors which condition the responses of mammals to rapid decompression and recompression.
111	University of California	John H. Lawrence Joseph G. Hamilton	Problems concerned with aeroembolism with the aid of radio-argon (110-minute and 37-day half-lives) and radionitrogen.
113	University of Chicago	Henry T. Ricketts	Effects of prolonged or repeated anoxia and low barometric pressure on certain aspects of adrenal, cardiovascular, renal, and nervous physiology, and studies of physical and psychologic fitness after simulated transportation by air.
121	Cornell University	Joseph C. Hinsey	Crash safety research.

Contract OEMcmr-	Contractor	Investigator	Subject
129	Mayo Clinic	Walter Boothby E. J. Baldes C. F. Code	Developing and testing equipment for oxygen supply and problems regarding centrifugal force, aeroembolism, liquid oxygen, dark adaptation, and other problems of aviation medical research.
130	New York University	J. Murray Steele	Relation of carotid sinus and carotid body sensitivity to changes in oxygen tension and barometric pressure in man.
133	University of California	Herbert M. Evans	Effects of adrenocorticotropic hormone (ACT) on the relation of adrenocortical physiology to the body's adaptation to low oxygen and low atmospheric pressure and to strains in general.
134	University of California	Edward S. Sundstroem	Role of the adrenal cortex in regulating low-pressure tolerance and the means that may be available for improving such a tolerance and for predicting its decline.
143	Massachusetts Memorial Hospitals	Robert W. Wilkins	Cardiovascular-renal responses to various stresses encountered in air warfare, especially "G."
147	University of Rochester	Wallace O. Fenn	Effects of high and low pulmonary pressures on the circulation.
163	Stanford University	Frederick A. Fender Henry W. Newman	Electroencephalographic studies in naval aviation inductees.
165	Harvard University	William G. Lennox	Relationship between brain metabolism and brain function and compensation for oxygen lack by changes in other blood constituents.
166	Princeton University	E. Newton Harvey	Gas bubble formation in blood, spinal fluid, lymph, and tissues as a result of pressure reduction.

Contract OEMcmr-	Contractor	Investigator	Subject
167	Harvard University	Frederic A. Gibbs	Electroencephalographic selection of air force personnel.
174	Harvard University	George W. Thorn	Relation of the adrenal cortex to anoxia.
182	University of Rochester	Karl U. Smith	Studies to determine the change in performance as illumination is decreased to scotopic levels and to discover the need to supplement adaptometer tests of night vision with tests of a psychomotor type.
191	Columbia University	Selig Hecht	Influence of altitude (or oxygen percentage) on the differential threshold (or brightness discrimination) of the eye.
193	Stanford University	L. R. Blinks, D. M. Whitaker, V. C. Twitty	Effects of decompression on cells, tissues, small blood vessels, blood, and tissue fluids, together with an analysis of factors affecting bubble formation, agglutination, and clotting.
196	University of California	John H. Lawrence, Joseph G. Hamilton	Physiological reactions in high-altitude flight, and the development of protective devices for the Army and Navy Air Forces that will increase the safety and efficiency of flying personnel.
199	John B. Pierce Foundation	L. P. Herrington, Harold Lamport	Protective devices against effects of high degrees of acceleration.
209	University of Pennsylvania	Detlev W. Bronk, H. K. Hartline	Visual mechanisms in relation to aviation medicine and development of instruments to improve visual efficiency in air crews.
210	University of Pennsylvania	William C. Stadie	Oxygen toxicity.
217	Marquette University	P. F. Swindle	Possible relationship of intravascular agglutination of red blood cells to decompression sickness and chronic altitude sickness.

Contract OEMcmr-	Contractor	Investigator	Subject
224	Columbia University	Charles Glen King	Effects of low oxygen pressure on nutritional requirements.
235	Swarthmore College	Laurence Irving	Development and application of methods and procedures for analyzing respiratory conditions.
236	Northwestern University	A. C. Ivy	Exploration of the degree of vitamin C deficiency induced and of disturbance of the metabolism of the vitamin by mild anoxia and its correlation with acid-base disturbances and 17-keto steroid excretion.
239	John B. Pierce Foundation	C. E.–A. Winslow	Development of radiation-insulated flight clothing.
241	Northwestern University	A. C. Ivy A. J. Atkinson	Effect of diet and drugs on aeroembolism.
257	Johns Hopkins University	Philip Bard	Physiological investigation of causes and nature of motion sickness in experimental animals.
258	University of Southern California	Paul O. Greeley	Reaction of animals to rapid and explosive decompression with respect to low oxygen tensions and low temperatures.
262	Duke University	Richard S. Lyman	Motion sickness, and construction of apparatus or equipment capable of producing artificial motion sickness.
264	Columbia University	Carl J. Warden	Motion acuity under scotopic conditions at various retinal positions.
267	Temple University	Ernest Spiegel	Prevention and treatment of motion sickness.
273	University of Southern California	Gordon H. Scott	Capillary circulation in animals exposed to the combined effects of high altitudes and low temperatures.
278	University of Minnesota	James J. Ryan	Design and construction of an airplane pilot's chair.
279	Wesleyan University	G. R. Wendt	Incidence, mechanisms, and therapy of motion sickness in young adult men.

Contract OEMcmr-	Contractor	Investigator	Subject
282	California Institute of Technology	David B. Tyler	Motion sickness and physiological problems peculiar to parachutists and other airborne troops.
288	University of Southern California	Douglas R. Drury Gordon H. Scott	Aviation physiology, with particular attention to the effects of acceleration, decompression, anoxia, and cold and methods to combat these effects.
289	Columbia University	Selig Hecht	Testing of color vision in relation to the color requirements of the Army and Navy.
290	University of Chicago	Melvin H. Knisely	Reactions of the peripheral vascular system aimed at prevention or alleviation of high-altitude decompression sickness.
291	Stanford University	F. W. Weymouth	Visual perception of distances as affected by illumination and other external conditions, with special reference to the lighting of landing fields and carrier decks.
306	University of Rochester	Wallace O. Fenn H. A. Blair	Abdominal gas in high-altitude flying.
339	Harvard University	Eugene M. Landis	Quantitative and comparative studies on effects of available anti-"g" garments on cephalic blood supply in man, using 1) the tilt table, 2) hot and cold environments, and 3) if requested by the services, the centrifuge at Wright Field or planes.
345	University of North Carolina	Frank N. Low	A test for peripheral visual acuity.
380	University of Pennsylvania	H. C. Bazett	Modification of oxygen equipment already designed for extreme altitude work to allow its use to aid escape from planes sinking in deep water (up to 100 feet).

Contract OEMcmr-	Contractor	Investigator	Subject
392	Massachusetts Institute of Technology	Robley D. Evans	Development of the technic for measuring saturation and desaturation curves by the use of long-period radioactive argon at atmospheric pressure.
414	University of California	William J. Kerr	Motion sickness.
421	Yale University	John F. Fulton	Compilation of a supplement to the classified bibliography of aviation medicine which was compiled under Contract No. OEMcmr-19.
429	Columbia University	Selig Hecht	Means of improving the selection, training, and employment of personnel for military duties involving visual operations, and manufacture and supply of approximately 100 Hecht visual contrast-discrimination test charts.
498	Yale University	John F. Fulton	Compilation of a bibliography of visual references.
524	University of Rochester	G. R. Wendt	Conditions and mechanisms of motion sickness.
435	Northwestern University	A. C. Ivy	Testing of the "Stratolator" for the Bureau of Aeronautics, Navy Department.
545	Harvard University	George Wald	Factors which govern the change in focus required by optical instruments when used at low and high illuminations.
559	Loyola University	Balint Orban	Cause and prevention of toothache under decompression; cause of gingival disturbances under decompression; causes of pain in recent amalgam fillings; action of zinc oxide eugenol fillings; and tissue changes in teeth extracted in decompression chamber.

Contract OEMcmr-	Contractor	Investigator	Subject
560	Mayo Clinic	E. J. Baldes	Human tolerance during high linear deceleration and to forces developed in aircraft crashes.

DIVISION OF PHYSIOLOGY

Blood, Blood Derivatives, and Blood Substitutes

40	Michael Reese Research Foundation	Sidney O. Levinson	To procure approximately 2,000 pints of human blood and to process such blood into plasma.
44	Bryn Mawr Hospital	Max M. Strumia	Preparation and preservation of plasma and blood substitutes.
69	Armour and Company	J. D. Porsche	To process and prepare human albumin from plasma furnished by the Government from approximately 2,000 pints of human blood, and to carry out sterility tests on plasma furnished by the Government and sterility and safety tests on solutions prepared therefrom and intended for intravenous use.
128	University of Tennessee	L. W. Diggs	Comparative efficiency of various types of equipment and various technics used in the collection of blood for transfusion and the development of technic using the milk bottle as a blood-transfusion flask.
132	Michael Reese Research Foundation	Sidney O. Levinson	Rapid desiccation of plasma and other substances.
139	Harvard University (Superseded OEMcmr-22)	Edwin J. Cohn	Production of sufficient amounts of plasma fractionation products to permit evaluation by clinical trial of the usefulness and proper methods of employment of these agents.
142	University of Wisconsin	J. W. Williams	Fractionation of proteins of human or animal plasma.

Contract OEMcmr-	Contractor	Investigator	Subject
151	Princeton University	Arthur K. Parpart	Influence of antioxidants on prolonging the stored life of red cells.
153	California Institute of Technology	Linus Pauling Dan H. Campbell	Chemical treatment of protein solutions in the attempt to find a substitute for human serum for transfusions.
162	Harvard University	F. H. L. Taylor G. R. Minot	Stability of certain labile plasma constituents with special reference to the preservation of plasma.
179	Stanford University	J. Murray Luck	Preparation of blood proteins and studies on serum albumin and products of plasma fractionation.
208	E. R. Squibb and Sons	H. B. van Dyke	Use of animal proteins as substitutes for human plasma or albumin.
219	University of Minnesota	Owen H. Wangensteen	Clinical testing of bovine albumin and metabolic-nitrogen studies on patients receiving bovine albumin.
237	Columbia University	David Seegal	Clinical and immunochemical testing of bovine albumin B.
238	Columbia University	Hans T. Clarke Edgar G. Miller	Chemical analysis of plasma fraction proteins and blood substitutes.
247	Armour and Company	J. D. Porsche	Experimental production of crystallized bovine serum albumin.
268	Stanford University	Thomas Addis	Studies to determine whether hydrolyzed pectin (proposed as a possible substitute for blood plasma) has any hitherto unrecognized deleterious effects when administered parenterally.
271	Massachusetts Institute of Technology	Hans Mueller	Optical properties of albumin and globulin solutions in relation to their stability.
330	Long Island College of Medicine	Jean R. Oliver	Pathologic effects produced in the kidney and other organs by blood substitutes.

Contract OEMcmr-	Contractor	Investigator	Subject
338	Stanford University	Thomas Addis	Physiological and structural effects of proposed blood substitutes, with special reference to renal effects.
372	Massachusetts Memorial Hospitals	Joseph F. Ross	In vitro preservation and post-transfusion survival of erythrocytes.
381	Children's Hospital, Cincinnati	Samuel Rapoport	Evaluation of different methods of preserving whole blood by studies of biochemical changes occurring in stored cells, with especial attention to the glycolytic enzymes and to their role in preserving the functional integrity of the cells.
384	Harvard University	Louis K. Diamond	To make available to the armed forces an adequate supply of Rh typing serum and to improve Rh typing material and typing method by concentrating the anti-Rh hemagglutinins from collected pools of suitable human serum.
409	University of Pennsylvania	John S. Lockwood	Enhancement of ossein gelatin as a plasma substitute through addition of amino acids and bovine serum ultrafiltrate.
453	Harvard University	Cutting B. Favour	Preservatives for biologic products, especially blood products.
471	Jewish Hospital of Brooklyn	Alexander S. Wiener	Preparation of anti-Rh typing sera.
482	University of Pennsylvania	Jonathan E. Rhoads W. M. Parkins	Physiological properties of globin, serum albumin, gelatin, and plasma.
509	New York City Cancer Institute	Johannes Vogelaar	Analysis of the mechanism of cell conglutination, as observed after infusion of various blood substitutes, with the purpose of developing a method to prevent it.

Contract OEMcmr-	Contractor	Investigator	Subject
532	Scripps Memorial Hospital (Cancelled — no work was done)	Eaton M. MacKay	Choice of anions in sodium salt solutions for therapy in postoperative dehydration, shock, and burns.
537	California Institute of Technology	Carl G. Niemann	Isolation of purified blood group specific substances from erythrocytes and gastric mucosa.
547	Boston University	William C. Boyd	Preparation of pure blood group A substance to serve as a standard of potency.
552	Columbia University	Elvin A. Kabat	Purification, characterization, and standardization of blood group substances (A and B).

Shock

3	New York University	Robert Chambers	Micromanipulative studies of capillaries in relation to shock.
5	Princeton University	Wilbur Willis Swingle	Role of certain adrenal steroids in the prevention and treatment of shock.
6	Johns Hopkins University	Alfred Blalock Henry N. Harkins Philip B. Price George W. Duncan	Clinical study of shock and a study of the capillaries in experimental shock.
8	University of Chicago	Dallas B. Phemister	Role of vasodepressor nerve stimulation in the production of shock.
10	Medical College of Virginia	Everett Idris Evans	Relative value of anesthetics in clinical shock.
11	Vanderbilt University	Paul D. Lamson	Carbohydrate metabolism in shock, with special reference to anesthetics.
12	University of Rochester	Stafford L. Warren	Temperature in relation to shock.
15	Harvard University	Henry K. Beecher	Relationship of anesthesia to shock.
16	Pennsylvania Hospital	Walter Estell Lee	Value of adrenocortical hormones in the treatment of shock due to burns.
21	Harvard University	Jacob Fine	Shock and the use of radioactive elements in the study of shock.

Contract OEMcmr-	Contractor	Investigator	Subject
66	Columbia University	Magnus I. Gregersen	Traumatic shock and the construction of approximately fifteen photometers.
67	Rockefeller Institute	Donald D. Van Slyke	Relationship between shock, crush injuries, and kidney functions.
104	Princeton University	Wilbur Willis Swingle	Effect of adrenal steroids in prevention of shock.
107	Columbia University	Dickinson W. Richards, Jr. Andre Cournand	Circulation of the blood during a period of shock and the effects of therapy thereon.
115	Carnegie Institution of Washington	George W. Corner Alfred Gellhorn Louis B. Flexner	Determination of the rate of escape of radioactive sodium from the blood stream in various stages of traumatic shock and following shock therapy.
124	Henry Ford Hospital	Henry Nelson Harkins	Fate in the body of blood substitutes used in the treatment of shock.
125	Eli Lilly and Company	Irvine H. Page	Crush injuries.
127	University of Nebraska	J. E. M. Thomson	Local shock.
131	Massachusetts Institute of Technology	Soma Weiss Robley D. Evans John G. Gibson, II Joseph C. Aub	Development of a method for determining the total volume of red cells by means of radioactive iron and application thereof to problems related to blood transfusion and shock.
135	Massachusetts General Hospital	Joseph C. Aub Austin M. Brues Waldo E. Cohn Ira T. Nathanson Paul C. Zamecnik	Traumatic shock, and more particularly the significance of fluid shifts and changes in cell permeability in initiating and sustaining the shock state.
144	Emory University	Eugene A. Stead, Jr.	Capillary permeability in shock, infection, and anoxemia.
145	Harvard University	Henry K. Beecher	Relationship of anesthesia to shock in the circulatory system.

Contract OEMcmr-	Contractor	Investigator	Subject
146	University of Rochester	George H. Whipple	Shock as influenced by blood plasma protein loss into and replenishment from the body tissues and factors governing this protein exchange.
172	University of Wisconsin	J. A. E. Eyster W. J. Meek	Experimental traumatic shock and the possible involvement of the heart in the shock picture.
201	University of Rochester	John J. Morton Earle B. Mahoney	A clinical and laboratory study of patients in shock from hemorrhage, trauma, and burns, with special relation to fluid and protein shifts, and a study of the role of infection in experimental shock.
203	University of Rochester	Stafford L. Warren	Distribution of body fluids and the possibility of a toxic factor in shock produced by crushing injury in the dog.
233	Harvard University	Lewis Dexter	Significance and possible therapeutic application of the renal humoral pressor mechanism in shock.
250	University of Rochester	Robert W. Ramsey	Shock in rats.
301	Michael Reese Hospital	Samuel Soskin	Cause and prevention of the so-called irreversible stage in shock.
327	Cornell University	Ephraim Shorr	A study, by in vitro methods, of the metabolic disturbances in the tissues of dogs after tourniquet shock, and the development of more satisfactory blood substitutes by the addition of suitable substrates or enzymes, capable of correcting these metabolic defects.
341	Yale	Milton C. Winternitz C. N. H. Long Ernest Mylon Alfred E. Wilhelmi	Shock.

Contract OEMcmr-	Contractor	Investigator	Subject
346	Emory University	Eugene A. Stead, Jr. James V. Warren	Shock and the dynamics of blood flow.
361	University of Wisconsin	Roland K. Meyer Van R. Potter	Biochemical studies on the shock problem, designed to develop rational therapeutic measures.
458	Mount Zion Hospital	Myron Prinzmetal Karl F. Meyer	Pathogenesis and treatment of shock due to muscle crushing.
536	Cleveland Clinic Foundation	Irvine H. Page	Renal syndrome of shock.

Nutrition, Acclimatization, and Miscellaneous Physiological Studies

27	University of Minnesota	Ancel Keys	Starvation and nutritional rehabilitation in man with relation to calories, protein, and vitamin therapy.
33	Harvard University	Shih Lu Chang	Destruction of the cysts of *Endamoeba histolytica* in drinking water.
46	Northwestern University	A. C. Ivy R. H. Seashore	Effect of benzedrine, pervitin, and caffeine on staying awake and on performance after a fatiguing march or after long flights.
54	Harvard University	Arlie V. Bock W. H. Forbes	Laboratory and field studies of fatigue in relation to environmental conditions, especially variations in temperature, diet, and altitude.
71	Northwestern University	Chester J. Farmer Arthur F. Abt	Methods for detection of early signs of vitamin C deficiency.
72	Northwestern University	A. C. Ivy	Effect of benzedrine, pervitin, and caffeine on the maintenance of efficiency under ordinary conditions and conditions of anoxia.
110	Mount Sinai Hospital	Harry Sobotka	Synthesis of vitamin A.

Contract OEMcmr-	Contractor	Investigator	Subject
122	Stanford University	Craig Taylor	Validation of the cardiovascular-respiratory fitness test (Taylor) as an index of physiological condition for aviation training and duty and study of circulatory responses on the tiltboard relative to tendency to blackout and general fitness.
180	Massachusetts General Hospital	Allan Butler	Toxicity and excretion of the constituents of ingested sea water and the treatment of dehydration with a minimal ingestion of fresh water.
184	Marine Studios	W. Douglas Burden	Possibilities of protection against sharks, barracuda, and jellyfish for men adrift in life belts.
188	Massachusetts Institute of Technology	Bernard S. Gould	Serum phosphatase in scurvy, the possible detection of subclinical scurvy, and the assay of ascorbic acid by the phosphatase level in experimental scurvy; and collagen formation as influenced by ascorbic acid.
192	University of Minnesota	Maurice B. Visscher	Effects of clothing on the yield of water by the body heat vacuum distillation method; preparation of potable water from sea water; and development of a lightweight, compact, practically nonbreakable apparatus therefor.
194	Harvard University	Hallowell Davis	Physiological effects of loud sounds, with special reference to traumatic deafness.
206	University of Rochester	Edward F. Adolph	Effects of dehydration and relief of thirst in desert troops.
218	New York University	Norman Jolliffe	Effect of light on production of corneal vascularization and its relation to riboflavin deficiency.

Contract OEMcmr-	Contractor	Investigator	Subject
220	University of Minnesota	Ancel Keys	Effect of high temperature at both high and low humidities on vitamin and mineral requirements of man, particularly military personnel.
222	University of Cincinnati	Robert A. Kehoe	Method for the quantitative determination of oxides of nitrogen (essentially NO_2) in presence of ammonia, hydrogen, and carbon monoxide.
225	University of California	Agnes Fay Morgan	Effect of dehydration on quality and nutritive value of foods.
227	University of Illinois	H. H. Mitchell	Effects of high temperatures and varied humidities on the vitamin requirements of man, with particular reference to the loss of water-soluble vitamins in perspiration under humid and arid tropical conditions; and vitamins and minerals in human perspiration and their significance with reference to requirements in tropical climates.
232	University of Michigan	Jerome W. Conn Margaret W. Johnston	Improvement of the ability of a soldier to work in humid heat.
234	University of Illinois	Robert W. Keeton H. H. Mitchell	Effect of specific dietary factors, including carbohydrates, proteins, fats, and vitamins, on the ability of the human body to withstand repeated exposure to intense cold.
240	University of Tennessee	Lathan A. Crandall, Jr.	To determine what diet is most satisfactory as a preparation for a period of starvation or reduced caloric intake.
244	Vanderbilt University	John B. Youmans (Cancelled — work never got under way)	Effect of glare from natural sunlight, artificial light, or both on the local and general requirements, the excretion or loss by other means, and the local or general manifestations of such loss of members of the B group of vitamins, especially riboflavin.

Contract OEMcmr-	Contractor	Investigator	Subject
246	Cornell University	Faith Fenton	Determination of the losses of nutritive value of foods through processing and cooking to ascertain whether vitamin supplements are needed for our overseas forces.
248	Public Health Research Institute of City of New York	Otto A. Bessey	Relation of riboflavin to injuries of the eyes.
249	University of Texas (Cancelled — work was never begun)	Jet C. Winters	Determination of vitamin losses in the dehydration of food.
251	Harvard University	Gordon M. Fair	Disinfection of water and related substances with special reference to the disinfection of water in canteen and Lyster bag quantities.
252	University of Michigan	Floyd E. Bartell	Development of fabrics with desirable surface properties and development and utilization of preparations of hydrophobic silica aerogel, with particular reference to their use as insulating material in fabrics.
261	University of Pittsburgh	Herbert E. Longenecker	Degree of digestibility for high melting point fats.
269	University of Michigan	A. C. Furstenberg	Human responses to cold, heat, and humidity, and improvement of protective clothing.
303	University of Texas	Roger J. Williams	Folic acid.
304	Textile Foundation	Milton Harris Lyman Fourt	Relation of clothing to the evaporative mechanism for retaining body temperatures.
312	Harvard University	Arlie V. Bock	Relative protein requirements of men doing hard physical labor.
313	Harvard University	Arlie V. Bock	Vitamin C requirements of men doing hard physical labor.

Contract OEMcmr-	Contractor	Investigator	Subject
328	Harvard University	Arlie V. Bock	Physiological adaptations of man to heat.
351	University of Indiana	Sid Robinson	Physiological effects of clothing on men performing work and at rest in hot climates; and effects of acclimatization on performance and clothing requirements in the heat.
364	Massachusetts General Hospital	Allan M. Butler	Emergency lifeboat and life raft provisions and reduction of insensible water loss by reducing absorption of heat.
365	University of Pennsylvania	L. V. Heilbrunn	Methods of protection against the adverse physiological effects of heat.
366	Stanford University	Arthur C. Giese Julian M. Wells	Sunburn protection.
373	Northwestern University	T. E. Friedemann A. C. Ivy E. E. Foltz	Utilization of riboflavin at low, intermediate, and optimum levels of intake and its relation to the work output of subjects of military age at ordinary atmospheric pressure and at high altitudes (15,000 feet).
376	University of Rochester	John R. Murlin	The use of eight or ten essential amino acids for determination of the biologic value of mixed proteins in adult human nutrition.
379	New York University	Norman Jolliffe	Detoxication of trinitrotoluene (TNT) by animal tissues and studies on the mechanism of its toxic action.
385	Duke University	William A. Perlzweig	Development of rapid methods of estimating relative saturation with, or depletion of, vitamins of the B complex, suitable for use in mass nutrition surveys.
387	Harvard University	Alan R. Moritz	Thermal (cold) injury of the lungs and air passages.

Contract OEMcmr-	Contractor	Investigator	Subject
425	Cornell University	N. S. Moore L. A. Maynard C. O. Mackey	Effect of protein level in the diet on resistance to cold, with special reference to the respiratory exchange and the reaction of the body thermostat at low temperatures.
430	University of Southern California	Frederick J. Moore John F. Kessel	Sterilization of water in canteens.
434	University of Oregon	Norman A. David E. S. West	Acute and chronic effects of sulfited foodstuffs (sulfur dioxide) in animals and man.
443	Columbia University	David E. Green	Fundamental biochemistry of disinfection.
474	Pentagon Post Restaurant Council	Fred G. Koch	Studies to determine the nutrient content of foods served in a large-scale operation, to determine the stages in the preparation of foods at which the loss in nutrients is greatest, and to devise means whereby these losses may be minimized by practical revisions in restaurant practice.
483	University of Rochester	Edward F. Adolph	Acclimatization to heat and cold.
506	Milton Harris Associates	Milton Harris Lyman Fourt	Influence of clothing on evaporation.
562	Public Health Research Institute of City of New York	Otto A. Bessey	Development of field methods for evaluating nutritional status.
M–1013	United States Department of Agriculture	I. P. Earle	Reduction in the bulk and weight of the ration for Army horses, with special reference to the minimum roughage requirements.
M–1573	United States Department of Agriculture	Esther L. Batchelder	Conservation of nutritive values of fruits, vegetables, and cereal grains, with special reference to losses during marketing, cooking, or home preservation.

DIVISION OF CHEMISTRY

Treatment of Gas Casualties

Contract OEMcmr-	Contractor	Investigator	Subject
9	University of Pennsylvania	Francis H. Adler	Effects of toxic agents on the eye.
13	University of Pennsylvania	I. S. Ravdin John S. Lockwood H. Davis Bruner	Lung irritants and the effectiveness of certain therapeutic agents.
24	Johns Hopkins University	Jonas S. Friedenwald Alan C. Woods	Vesicant injuries of the eye, especially those caused by war gases.
39	Yale University	M. C. Winternitz Henry Bunting Harold E. Harrison	Lung irritation produced by toxic agents and toxicity of various therapeutic agents in the treatment of gas casualties.
51	Yale University	William T. Salter Ashley W. Oughterson Harry M. Zimmerman	Mode of action and therapy of chemical irritants.
57	University of Chicago	E. S. Guzman Barron	Biochemical effects of war gases and insecticides on enzyme tissues.
73	Northwestern University	A. C. Ivy	Therapy of phosgene poisoning.
82	Johns Hopkins University	Maurice Sullivan	Relation of nutritional deficiencies to the action of vesicants on the skin.
83	Yale University	Samuel C. Harvey Gervase J. Connor	Promotion of healing in the residual lesions after primary treatment of tissues exposed to vesicant gases.
92	Saranac Laboratory	Leroy U. Gardner A. J. Vorwald	Possible chronic diseases of the lungs and other organs due to repeated exposures to chemical-warfare agents and the influence of such exposures on susceptibility to tuberculosis.
96	Memorial Hospital, New York	C. P. Rhoads	Hemolytic effects of organic and inorganic compounds, the mechanism of the hemolysis, and methods of protecting cells against hemolysis.

Contract OEMcmr-	Contractor	Investigator	Subject
97	May Institute	I. Arthur Mirsky	To devise therapy based on fuller knowledge concerning the role of the nervous system in the reaction of chemical-warfare agents.
98	May Institute	I. Arthur Mirsky	Mechanism of delayed reactions and treatment of burns of the eye due to chemical agents.
101	Columbia University	Karl Meyer	Mode of action of vesicant gases on the cornea, and treatment of gas burns.
103	Cornell University	David P. Barr Marion B. Sulzberger John M. McLean	Effect of chemical agents on the skin.
108	University of Pennsylvania	I. S. Ravdin D. Wright Wilson D. M. Pillsbury Harry M. Vars	Healing of cutaneous and other lesions produced by toxic agents, and food contamination with various toxic agents and their possible decontamination.
114	University of Chicago	Ralph W. Gerard	Neutralization of pulmonary irritants.
141	Harvard University	David G. Cogan	Effect of vesicant agents under various conditions on the permeability of the cornea, conjunctiva, and sclera, and a micro test for HS.
152	University of Chicago	William Bloom	Treatment of chemical injuries to the skin.
245	Cornell University	McKeen Cattell	Pharmacology of new toxic agents.
253	Johns Hopkins University	Warfield T. Longcope	Gas casualties.
507	Columbia University	Michael Heidelberger Elvin A. Kabat	Immunochemical studies.
M-2214	Food and Drug Administration	Herbert O. Calvery	Physiological properties of preparations such as BAL solutions and ointments and other compounds which may be developed for use in the prevention and treatment of gas casualties.

Insect and Rodent Control

Contract OEMcmr-	Contractor	Investigator	Subject
29	Gorgas Memorial Institute	Daniel M. Jobbins Herbert C. Clark	Insect repellents as a basis for the development of better preventive measures.
59	Johns Hopkins University	Curt P. Richter	Poison for rats, ground squirrels, and other wild rodents.
296	University of Pennsylvania	A. Glenn Richards, Jr.	Insect repellents, and more particularly their method of penetration into the nervous system and their mode of action in relation to their practical use.
375	Ohio State University	Dwight M. DeLong Ralph H. Davidson	Action of insect repellents in terms of their effects on insect behavior and in relation to their properties, thereby to facilitate selection of new compounds for practical repellent tests.
394	City of Baltimore		Reports on the progress of a city-wide campaign of rat extermination, using a rat poison developed under Contract OEMcmr-59.
416	E. I. du Pont de Nemours and Company		Manufacture of ANTU (alpha-naphthylthiourea), a rat poison.
523	Harvard University	John H. Welsh	Mode of action of insecticides, especially DDT.
529	New York University	Daniel Ludwig	Physiological action of DDT and other insecticides.
531	Tufts College	Kenneth D. Roeder	Action of toxic substances on the insect central nervous system.
538	University of California	Tracy I. Storer Milton A. Miller	Preparation of a bibliography of rodent control.
546	University of California	Roderick Craig W. M. Hoskins	Mode of action of dichloro-diphenyl-trichloroethane (DDT) as an insecticide.
548	Mt. Sinai Hospital	Joseph H. Globus	The nervous system in animals suffering from chronic and acute DDT poisoning.

Contract OEMcmr-	Contractor	Investigator	Subject
555	Gorgas Memorial Institute	Herbert C. Clark	Insect repellents as a basis for the development of better preventive measures.
557	University of Minnesota	A. Glenn Richards, Jr.	Physiological action of insecticides and insect repellents.
M–2218	Food and Drug Administration	Herbert O. Calvery John Draize	Possible toxicity of substances proposed for use as insect repellents and lousicides.
M–3167	Fish and Wildlife Service	E. R. Kalmbach	To find and develop new rodenticides for use in controlling rats and injurious field rodents.
M–4331	United States Department of Agriculture	P. N. Annand W. E. Dove F. C. Bishopp E. C. Cushing	Methods of control for insects affecting the armed forces.
M–5251	Food and Drug Administration	Herbert O. Calvery Geoffrey Woodard	Toxicity of compounds in use and proposed for use in the control of rodents.
M–5356	Food and Drug Administration	Herbert O. Calvery John Draize	Primary irritation of proposed insect repellents.

MALARIA

Contract OEMcmr-	Contractor	Investigator	Subject
4	University of Michigan	L. T. Coggeshall Richard J. Porter	Chemotherapy of malaria.
7	University of Tennessee	Arthur P. Richardson Reginald I. Hewitt Marion Brooke	Chemotherapy of malaria.
49	Emory University	Elizabeth Gambrell	Prophylactic and therapeutic effects of new drugs in malaria.
58	Gorgas Memorial Institute	Carl M. Johnson	Serodiagnostic methods in malaria.
77	University of Chicago (Superseded OEMcmr-2)	W. H. Taliaferro E. M. K. Geiling Earl A. Evans, Jr. C. G. Huff	Chemotherapy of malaria.
90	Johns Hopkins University	E. K. Marshall, Jr.	Chemotherapy of malaria.
91	University of Tennessee	F. L. Roberts	Immunologic reactions in malaria.

Contract OEMcmr-	Contractor	Investigator	Subject
112	New York University	James A. Shannon Donal Sheehan David P. Earle	Relationship between sustained plasma concentration and the therapeutic effectiveness of quinine, quinine substitutes, and new potential antimalarial drugs in therapeutic malaria in man, and clinical and field tests of antimalarials.
173	Harvard University	Frederick J. Stare	Interrelationships between nutrition, malaria, and the action of antimalarial drugs.
186	Johns Hopkins University	F. Y. Wiselogle W. Mansfield Clark Alan M. Chesney Donald H. Andrews	Facilitation of efforts to obtain an effective antimalarial.
187	Columbia University	Enid T. Oppenheimer Barry G. King	Effect of atabrine on normal animals at sea level and at altitudes, with emphasis on toxic factors and their influence on altitude tolerance.
200	University of Virginia	Robert E. Lutz	Synthesis of antimalarials.
212	California Institute of Technology	Joseph B. Koepfli Edwin R. Buchmann	Chemistry of potential antimalarials.
223	University of California	Chauncey D. Leake	To ascertain and, if possible, devise protection against potential toxic reactions of antimalarial drugs on long-continued, frequently repeated administration (as in prophylactic use), and to devise chemical and biologic tests to determine whether or not accidental or malicious contamination of such drugs has occurred.
226	Woman's Medical College of Pennsylvania	Ben King Harned	Influence of quinine and atabrine on the absorption, conjugation, and excretion of the sulfonamides commonly used in therapeutics.
242	Harvard University	Louis F. Fieser	Synthesis of antimalarials.

Contract OEMcmr-	Contractor	Investigator	Subject
256	Columbia University	Michael Heidelberger	Antigenic properties of human malarial parasites.
260	University of California	T. E. Weier S. H. Babcock H. A. Young	Search for antimalarials among native plants of western states.
266	Merck Institute	Hans Molitor	Chronic toxicity of atabrine.
274	Columbia University	Heinrich Waelsch	Metabolic products of atabrine in the animal body.
276	New York University	Kenneth Clark Blanchard	Nature of the end-products formed from atabrine in the animal body.
	(Cancelled — work was not started)		
277	Vanderbilt University	Paul D. Lamson	Determination of degradation products of atabrine in the body (animals and man).
284	Duke University	Charles R. Hauser	Synthesis of new antimalarial side chains.
297	Johns Hopkins University	W. W. Cort	Standardization of the "3T" strain of *Plasmodium cathemerium* in ducks for use in chemotherapy investigations and studies on simian and avian malaria.
298	Indiana University	R. L. Shriner D. G. Thomas J. H. Billman	Syntheses of organic compounds for use as antimalarials.
299	DePauw University	J. L. Riebsomer M. C. Kloetzel	Synthesis of organic compounds for use as antimalarials.
305	University of Notre Dame	Kenneth N. Campbell	Synthesis of possible antimalarials.
309	Dartmouth College	Elden B. Hartshorn	Synthesis of potential antimalarial drugs.
310	Northwestern University	Byron Riegel Robert H. Baker	Synthesis of organic compounds for use as potential antimalarial drugs.
314	University of Southern California	Ronald F. Brown M. C. Kloetzel	Synthesis of potential antimalarial drugs.
315	University of Maryland	Nathan L. Drake	Synthesis of certain substituted sulfanilanilides and certain substituted sulfadiazines and such other syntheses as may be required.
317	University of Chicago	E. M. K. Geiling W. H. Taliaferro	Chemotherapy of malaria.

Contract OEMcmr-	Contractor	Investigator	Subject
319	California Institute of Technology	Edwin R. Buchman	Synthesis of potential antimalarial drugs.
320	University of Tennessee	Robert Briggs Watson Henry Packer	Clinical testing of antimalarials.
321	University of Illinois	Charles C. Price	Synthesis of organic compounds for use as potential antimalarial drugs.
323	University of California	Thomas L. Jacobs	Synthesis of potential antimalarial drugs.
324	University of Missouri	H. E. French	Synthesis of organic compounds for use as antimalarials.
332	University of Minnesota	Walter M. Lauer	Synthesis of organic compounds for use as potential antimalarial drugs.
333	Cooper Union	Clarence S. Sherman	Synthesis of potential antimalarial drugs.
340	Columbia University	Robert C. Elderfield Walter J. Gensler	Synthesis and analysis of new antimalarial drugs.
342	Tulane University	Walter James Horton	Synthesis of potential antimalarials.
343	Stanford University	Francis W. Bergstrom	Synthesis of potential antimalarials.
344	Johns Hopkins University	Leslie Hellerman	Biochemistry of antimalarials.
350	Harvard University	Eric G. Ball Quentin M. Geiman	Metabolic characteristics of malaria parasites with special emphasis on their energy-yielding processes; that is, oxidative and phosphorylating mechanisms.
352	Elizabeth Gamble Deaconess Home operating Christ Hospital, Cincinnati	Leon H. Schmidt	Pharmacology and toxicity of antimalarial compounds.
353	University of Chicago	Earl A. Evans, Jr.	In vitro action of antimalarials on normal and parasitized (*Plasmodium gallinaceum*) chicken erythrocytes, with special reference to the factors involved in the distribution of drugs between parasitized cells and plasma.

Contract OEMcmr-	Contractor	Investigator	Subject
354	University of Michigan	F. F. Blicke	Antimalarials.
356	Oregon State College	Charles S. Pease	Synthesis of potential antimalarials.
357	Cornell University	William F. Bruce	Synthesis of new and potential antimalarial drugs.
358	Cornell University	Vincent du Vigneaud	Relationship of growth and anti-growth substances to the malarial organism.
368	Mt. Holyoke College	Mary L. Sherrill	Syntheses of new and potential antimalarial drugs.
377	Rockefeller Institute	R. W. Glaser	Malaria, with special reference to experimental hosts for the plasmodia which parasitize man.
405	University of Texas	Wendell Gingrich	Chemotherapy of malaria.
406	University of Tennessee	William B. Wendel	Influence of antimalarials on metabolism of plasmodia in vitro, and blood levels of antimalarial drugs.
412	Allied Chemical and Dye Corporation, National Aniline Division	Wesley Minnis	Preparation of approximately 200 pounds of 1,2,3,4-tetrahydrophenanthrene and semiworks studies on manufacture of said substance.
419	Massachusetts General Hospital	Allan M. Butler	Metabolic and therapeutic studies on synthetic antimalarial drugs.
423	University of Michigan	Maurice H. Seevers	Pharmacology and toxicology of antimalarial drugs.
440	Winthrop Chemical Company	M. L. Tainter	Synthesis of 10 kg. of 3-methyl-7-chloro-4-(1-methyl-4-diethylamino-butylamino)quinoline.
450	University of Chicago	Alf S. Alving	Pharmacology and clinical testing of antimalarial agents.
459	University of Michigan	Reuben L. Kahn	Serology of malaria.
467	E. I. du Pont de Nemours and Company	J. E. Kirby	Synthesis of compounds for testing as antimalarials.
475	Rockefeller Institute	Donald D. Van Slyke	Methods for determining antimalarial drugs.
481	E. R. Squibb and Sons	Arthur P. Richardson	Chemotherapy of malaria.

Contract OEMcmr-	Contractor	Investigator	Subject
486	University of Rochester	D. S. Tarbell	Chemical research on the problem of antimalarials.
488	Allied Chemical and Dye Corporation, National Aniline Division	Wesley Minnis	Preparation of 4200 pounds of 4 : 7-dichloroquinoline, the conversion of not more than 3000 pounds of the same into the finished product SN–7618, and a laboratory investigation and comparative evaluation of certain alternate syntheses of 4 : 7-dichloroquinoline.
503	Rockefeller Institute	Lyman C. Craig	Chemical investigation of antimalarial drugs.
516	University of Pennsylvania	Marvin Carmack	Synthesis of new antimalarial drugs.
530	Massachusetts Institute of Technology	Arthur C. Cope	Chemical research on the problem of antimalarials.
	(Cancelled before work was begun)		
539	Cornell University	John R. Johnson	Synthesis of large-membered ring compounds for antimalarial tests.
554	Sharples Chemicals, Incorporated	John F. Olin	Production of amines and halides as specified by formula for use as intermediates in antimalarial drugs.
556	Johns Hopkins University	John M. Chemerda	Synthesis of potential antimalarial drugs.
563	Northwestern University	C. D. Hurd	Synthesis of therapeutic agents and intermediates.
564	State University of Iowa	George H. Coleman	Synthesis of therapeutic agents and intermediates.
565	Iowa State College	Henry Gilman	Synthesis of therapeutic agents and intermediates.
566	University of Nebraska	C. S. Hamilton	Synthesis of therapeutic agents and intermediates.
567	University of Wisconsin	Homer Adkins	Synthesis of potential antimalarial agents and intermediates.
570	University of Illinois	R. C. Fuson	Synthesis of therapeutic agents and intermediates.
572	J. W. Edwards, Publisher		Publication of the monograph entitled "Survey of Anti-Malarial Drugs 1941–1945."

Contract OEMcmr-	Contractor	Investigator	Subject
M–487	National Institute of Health	Lyndon F. Small G. Robert Coatney W. H. Sebrell	Testing of antimalarial drugs.
M–488	National Institute of Health	Lyndon F. Small W. H. Sebrell	Synthesis of antimalarial drugs.
M–3993	National Institute of Health	G. Robert Coatney W. H. Sebrell	Prophylactic (suppressive) and therapeutic studies on mosquito-induced *Plasmodium vivax*.

ADRENOCORTICAL HORMONES

32	Princeton University	Everett S. Wallis	Synthesis of physiologically active hormones of the adrenal cortex, especially Compound E.
42	University of Chicago	T. F. Gallagher	Synthesis of cortical sterols.
52	St. Louis University	Sidney A. Thayer	Bioassay of available adrenocortical extracts.
55	Stanford University	Carl R. Noller	Synthesis of cortical hormones, especially Kendall's Compound E.
70	University of Virginia	S. W. Britton	Synthesis of physiologically active hormones of the adrenal cortex, especially compounds substituted on carbon atom 11 of the sterol nucleus (E type).
95	Wake Forest College	Arthur Grollman	Search for synthetic substitutes for the adrenocortical hormone.
106	Yale University	Werner Bergmann	The introduction of oxygen into the position 11 of the steroid ring system, and the preparation of compounds of the type of Kendall's substance E from readily accessible material.
168	Harvard University	Louis F. Fieser	Synthesis of cortical steroids.
292	Memorial Hospital, New York	C. P. Rhoads	The isolation from human urine of steroids which may serve as intermediates for the synthesis of adrenocortical steroids.

ANTIBIOTIC AGENTS

Contract OEMcmr-	Contractor	Investigator	Subject
100	Bradley Polytechnic Institute	Robert D. Coghill	Cultural methods and assay of penicillin.
119	Princeton University	Frank H. Johnson	To find a method by bacterial luminescence for quick titration of potency of penicillin preparations.
155	St. Louis University	Edward A. Doisy	Microbial antagonist organisms, a study looking toward the discovery and development of products superior to penicillin and pyocyanase.
275	Massachusetts Memorial Hospitals	Chester S. Keefer Donald G. Anderson	Collection of information concerning penicillin; clinical study of sulfamethyldiazine; and co-operation through consultation and otherwise as may be practicable with other persons and governmental or other agencies or organizations concerned with research, development, production, or use of penicillin.
389	E. R. Squibb and Sons		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
390	Charles Pfizer and Company		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
391	Merck and Company		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
396	Abbott Laboratories		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
397	Eli Lilly and Company		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
398	Parke, Davis and Company		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
399	The Upjohn Company		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.

Contract OEMcmr-	Contractor	Investigator	Subject
408	University of Michigan	Werner E. Bachmann	Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
410	United States Department of Agriculture	Robert D. Coghill Frank H. Stodola	Chemical structure of penicillin.
411	Cornell University	Vincent du Vigneaud	Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
428	Winthrop Chemical Company Heyden Chemical Corporation		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
439	University of Illinois	George L. Clark	Crystalline and molecular structures of penicillin and related natural and synthetic compounds.
442	University of Michigan	H. M. Randall	Infrared pictures of penicillin crystals.
445	Shell Development Company Cutter Laboratories		Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
465	Food and Drug Administration		Improvement of the assay procedures used in the control of the quality and purity of penicillin.
540	Harvard University	R. B. Woodward	Chemical structure of penicillin and synthesis of penicillin or a therapeutic equivalent.
542	Cornell University	John R. Johnson	Compilation of a chemical index and aid and advice in connection with the disposition of patent questions in the program for the chemical study of penicillin.
544	Merck Institute	Hans Molitor	Pharmacologic and toxicologic properties of streptomycin, with particular emphasis on the absorption, distribution in body tissues, and excretion of the drug in a variety of animal species.

Contract OEMcmr-	Contractor	Investigator	Subject
551	Washington University	W. Barry Wood, Jr.	Pharmacologic and toxicologic study of streptomycin.
553	St. Louis University	Edward A. Doisy	Discovery and development of products superior to penicillin and pyocyanase.
561	Stanford University	G. W. Beadle	Penicillin biosynthesis and inactivation in culture in relation to the genetic constitution of the organism.
569	Massachusetts Memorial Hospitals	Donald G. Anderson	To compile information concerning penicillin, analyze penicillin reports, and prepare material for a monograph on penicillin.
571	National Academy of Sciences	Hans T. Clarke	To prepare a monograph on work done under Government sponsorship on the structure and synthesis of penicillin or a therapeutic equivalent, and on similar work done under the auspices of the Government of the United Kingdom.

MISCELLANEOUS

23	National Academy of Sciences (Supersedes OEMcmr-1)		To conduct and have conducted, utilizing technical experts, committees, subcommittees, and other facilities available, or which it can make available, studies and experimental investigations and to prepare, duplicate, acquire and/or furnish reports on: chemotherapeutic and other agents, blood transfusion, clinical medicine, surgery, aviation medicine, neuropsychiatry, co-ordination of information on military medicine, industrial medicine, treatment of gas casualties, and other similar subjects, as may be agreed upon.

INDEX

339; bodily position, influence of, 338; circulatory failure, patterns of, 338; peripheral resistance, 337–338; renal vasoconstriction, 338; toxic factors, effects of, 339;

therapeutic agents, effects of: 337; albumin, 337; blood and plasma substitutes, 337; gelatin, 337; plasma, 337; saline solution, 337; whole blood, 337 *See also* Burns; Shock, experimental traumatic

Shock, experimental traumatic, 341–356; aim and scope of study, 341–342; anesthetics and analgesics, 352–353; capillary permeability, effect on, 342–343; from endogenous non-bacterial toxins, 348–349; liver in relation to shock, 349, 355–356; nervous system, role of, 347; temperature effects, 351–352; temperature effects, vasoconstrictor action of cold, 352; tourniquet compared to hemorrhagic shock, 349–351; from vasodepressor agent, 349;

hemodynamics, distortion of: 343–347; dye-plasma-hematocrit method, 343; tracing of red cells with radioactive iron, 344–345;

stagnation in peripheral vascular bed: 343–347; sequence of events, 346; from infection: 347–348; clostridia toxins, 348; *cl. welchii* toxin, 348;

irreversible shock, therapy of: 353–354; agents tested, 354; replacement of whole blood and plasma, 353;

tissue anoxia: 354–355; effect on kidney, 354–355; effect on liver, 355–356. *See also* Shock

Skin, blister-gas injuries, 588–602; late treatment of, 598–602. *See also* Blister gases

Skin grafting, fibrinogen and thrombin in, 430

Sleeping bags, testing of, against cold, 499–500

Sodium chloride, loss of, through exposure to heat, 492–493

Sodium glycerol polysuccinate, as synthetic blood substitute, 460

Spinasterol, investigations on, 660

Staphylococcus aureus, effect of penicillin on, 125–127

Starvation, dietary conditioning against, 480; nutritional aspects of, 479–480

Steroids, adrenocortical. *See* Adrenocortical steroids

Streptococcus infections, hemolytic. *See* Hemolytic streptococcus infections

Streptococcus MG, in primary atypical pneumonia, 22–23

Streptomycin, 16, 37, 118; for Asiatic cholera, 16; for plague, 37

Sulfadiazine, antimalarial activity of, 694; as causal prophylactic in *gallinaceum* malaria, 692; in experimental gas gangrene, 142–143; in wound infections, 98

Sulfamylon. *See* Marfanil

Sulfanilamide, for wound infections, 112–113

Sulfanilamide derivatives, as causal prophylactics in *gallinaceum* malaria, 692

Sulfathiazole, for chancroid, 47; for gonorrhea, 50; microcrystals in wound infections and burns, 108–109

Sulfonamides, for Asiatic cholera, 16; for bacillary dysentery, 7–8; for burns, 108, 150; for burns, infectious, 157; for burns, vesicant, in creams, 599; for chancroid, 47; for compound fractures, 103–106; for eye injuries, chemical burns of, 610; for eye injuries, experimental healing of, 120–121; for gas gangrene, 39, 42; gas gangrene, for experimental, 142–143, 143 (table); for gonorrhea, 48–50; for hemolytic streptococcus infections, 30; for malaria, 665–666; for nerve injury, 164; for plague, 37; for primary atypical pneumonia, 21; synergists, potentiators, and antagonists of, 115–119; for wound infections, 95–106, 113, 119. *See also* Sulfadiazine; Sulfanilamide; Sulfanilamide derivatives; Sulfathiazole

Superpalite, as respiratory irritant, 568

Surgery, application of penicillin to problems of, 123–133

Surgery of nerves. *See under* Peripheral nerve lesions

Surgery of wounds, 91–94; aims, 91–92; brain tissues, repair of, 93; burns and shock, 92–93; chemotherapeutic agents, 92; research, 94; requirements, 91; special problems, 93; wound ballistics, 93–94 (*See also* Wound ballistics)

Surgical infections, complications of, 124–125

Surgical plastics and hemostatics, 182–185; celluronic acid, 183; fibrin foam, 183; oxidized cellulose gauze, 184; thrombin, 182–183;

other substances: 184–185; gelfoam, 184; oxidized cellulose, 185

Sutures. *See* Collagen sutures

Sweat loss, from exposure to heat, 491–492; factor in protective clothing, 504–505

Syphilis, 46, 50–64; biologic false-positive serologic tests, 63–64; biologic false-